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A PROTEIN COMPONENT OF THE GOLGI APPARATUS

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STUDIES on the composition of the Golgi apparatus are marked by an emphasis on the lipid components of this structure. This is readily understandable since some lipids are rather readily demonstrated and since peculiarly specific conditions utilizing quasilipid methods often are required to visualize the Golgi apparatus in permanent preparations. Studies on possible protein components are so equivocal that Baker,[1] in 1944, was moved to write:

> . . . The suggestion that the Golgi element contains protein does not rest on strictly histochemical evidence. It is perhaps unlikely that any part of the cell is totally lacking in proteins, but histochemical tests for them have not been shown to give positive reactions in the Golgi element.

The chief evidence bearing on this subject is the stainability of the Golgi apparatus when Weigert's resorcinfuchsin, the iron-hematoxylin technic, aniline blue and nigrosin are used.

This paper is concerned with a description of what appears to be a carbohydrate-containing protein in the Golgi apparatus of several types of cells, primarily the columnar cells of the mucosa of the duodenum of the rabbit and the guinea pig. An attempt was made to characterize this substance by the use of solvents and enzyme preparations.

MATERIAL AND METHODS

Tissues were prepared by freezing and drying and were then embedded in paraffin. Sections were deparaffinized and were either coagulated with alcohol directly or after being treated with a reagent. They were then stained for polysaccharides according to the method suggested by Hotchkiss.[2] This procedure involves the oxidizing to aldehydes of certain alcohol groups of carbohydrates bound to tissue substance and visualizing the compound so formed as a red color by the use of the Feulgen reagent. When sections are coagulated with alcohol, the Golgi apparatus of the columnar duodenal cell appears clearly as a delicate

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1. Baker, J. R.: Quart. J. Micr. Sc. **85**:1, 1945.
2. Hotchkiss, R. D.: Arch. Biochem. **16**:131, 1948.

pink or red network differentiated from the just perceptibly pink or colorless supranuclear protoplasm. Only when a reagent results in marked diminution or complete absence of stainability of the Golgi apparatus is it considered that the reagent has taken part in removal of the reactive components.

It may be helpful at this point to supplement the discussion of Hotchkiss on the specificity of the periodic acid—leukofuchsin method under these conditions. Carbohydrates may occur free in sections of frozen-dried material or combined in the forms of a polysaccharide (glycogen), certain lipids (considered in detail in the following pages), possibly lipoproteins and glycoproteins. Freely diffusible reactive components pass into solution from the section in the various steps of the staining procedure. Glycogen may be removed by the use of saliva after protein coagulation. The following lipids (free) have been found to give a purple-red addition product with the Feulgen reagent after being oxidized with periodic acid: kerasin, phrenosin, phosphatidyl ethanolamine, acetal phosphatide, inositol phosphatide, lecithin and ganglioside.[3] All of the free lipids may be removed by prolonged extraction with a hot mixture of equal parts of chloroform and methanol. However, it is possible that some or all of these lipids may be combined as lipoproteins. The glycolipid-protein complex recently described by Folch and Uzman[4] might be expected to be extracted if the section is treated with hot methanol-chloroform for sixteen hours. It is possible that other lipoprotein complexes may not be extracted by the procedure employed and would remain in the section with enough appropriate reactive groups retained to give a red stain with the periodic acid—leukofuchsin reaction. In addition to these compounds, the only remaining substances which would give a positive red color would then be carbohydrate-protein complexes. The distinction between lipoproteins and glycoproteins may be clarified by further studies of the extractabilities of stainable components following the application of chemical reagents and enzyme preparations. Thus, if the stainable component shows an isoelectric point and other properties corresponding to those of a chemically isolated and known glycoprotein, then the stainable component may be identified rather clearly as such a compound. Again, if a stainable component is extracted after the application of hyaluronidase, it may reasonably be assumed to be a glycoprotein. However, in other instances, where less is known of the chemical nature of purified extracts or of the properties of the stainable cell components in sections, it may become difficult or impossible to distinguish clearly between, or identify, reactive groups as component parts of lipoproteins or of glycoproteins.

The details of the procedure used are as follows: Five normal adult rabbits and 6 normal adult guinea pigs were killed by a blow on the head. Very small snips of the cephalic portion of the duodenal mucosa were removed and immersed in isopentane at approximately —160C. After freezing they were dried in a vacuum at about —30C., infiltrated with paraffin (melting point, 56 to 58 C.) during fifteen minutes, and embedded. Sections 6 microns thick were cut and mounted with slight pressure on an albuminized slide. The section was warmed again until the paraffin was just melted and then was flattened with slight pressure. The standard of reference was prepared by removing the paraffin with xylene or benzin and coagulating the proteins by immersing the slide in absolute alcohol for about sixteen hours. The section was then stained by the alcohol periodic acid—leukofuchsin method described by Hotchkiss.[2] The staining procedure was performed in a uniform manner in all instances except one in which sections were stained without prior oxidation. Invariably, such sections

3. Hack, M. H.: Personal communication to the author.

4. Folch, J., and Uzman, L. L.: Federation Proc. 7:155, 1948.

were colorless or nearly so. The treating of the section with reagents prior to alcoholic coagulation and staining was varied in order to study some of the properties of the reactive material of the Golgi apparatus. Enzyme preparations were washed from the section with 5 or 10 drops of the buffer used to dissolve the enzyme, and the section was then coagulated with alcohol and stained as usual. With respect to the methods of treatment, the concentration, the time of action and the effect on the Golgi apparatus are given in the table.

Effects of Various Extractives and Enzyme Preparations on Golgi Apparatus of Columnar Duodenal Cells of Guinea Pig After Mucosa Had Been Prepared by Freezing and Drying, Sectioned and Treated by Periodic Acid—Leukofuchsin Method

Reagent	Concentration	Time of Action	Stainability of Golgi Apparatus*
Acetate buffer, p_H 3.6-5.0	M/5	1 hr.	+
Phosphate buffer, p_H 5.5-8.0	M/15	1 hr.	+
Borate buffer, p_H 8.5-11.0	M/5	1 hr.	+ weak
Acetic acid (p_H 3.3)	0.2%	1 hr.	Very weak
Acetic acid	2.0%, 10%, glacial	1 hr.	—
Hydrochloric acid	N/10	1 hr.	+
Acetic acid (10%) followed by hydrochloric acid (N/10)		1 hr. 1 hr.	+
Hydrochloric acid in 30% alcohol	N/10	1 hr.	+
Trichloroacetic acid	5%	1 hr.	+
Propionic acid		1 hr.	+
Ammonium sulfide	Saturated	15 min.	Weak
Ammonium hydroxide	.00001N	1 hr.	Very weak
Sodium hydroxide	.00001N	1 hr.	Very weak
Carbon disulfide		1 hr.	+
Pyridine		1 hr.	+
Dioxane		1 hr.	+
Amyl acetate		1 hr.	+
Isoamyl alcohol		1 hr.	+
Hot methanol-chloroform, 1:1		16 hr.	+
Saliva in buffer, p_H 7.0	1:4	1 hr.	+
Pepsin	0.1 mg. per cc. .01N HCl	5 min.	—
	0.2 mg. per cc. in phosphate buffer, p_H 8.0	5 min.	—
Pangestin	0.1 mg. + .2 mg. enterokinase per cc. phosphate buffer, p_H 8.0	5 min.	—
Trypsin	2 mg. per cc. acetate buffer, p_H 4.0 and 6.0	1 hr.	+
Emulsin	1 mg. per cc. phosphate buffer, p_H 7.0	1 hr.	+
Hyaluronidase		1 hr.	Very weak
Snail stomach fluid	1 cc. per 10 cc. phosphate buffer, p_H 7.0	8 hr.	Very weak
Cl. welchii toxin			

*A plus sign indicates that the Golgi apparatus was stainable; a negative sign that it was not stainable.

OBSERVATIONS

Sections of duodenum which were freed of paraffin and coagulated by alcohol show the maximum total amount of reactive groups, which appear more or less red according to their number in specific sites (figs. 1 to 5). The epithelium of the glands of Lieberkühn and of the villi is attached to a thin, rather uniform basement membrane. As in other sites studied (kidney, skin, thyroid gland, lung) this appears as a rather uniform, deeply stained homogeneous

membrane which is continuous with the fainter-staining ground substance of the lamina propria mucosae (figs. 2 to 5). The ground substance of the basement membrane infiltrates the network of reticular fibers which lie in it and which are related to those of the lamina propria. The columnar epithelial cell is separated from adjacent cells by thin, sharp membranes of cement substance, which appear as pink lines. The striated border is deeply stained. The colorless nucleus

Fig. 1.—Low power photomicrograph of a transverse section of duodenal glands of the rabbit. The specimen was fixed by freezing and drying, sectioned, and stained by the periodic acid—leukofuchsin method. *GA* indicates the Golgi apparatus; *SNG*, supranuclear granules; *CB*, the cuticular border; *N*, a nucleus; *CTS*, connective tissue stroma; *GC*, a goblet cell. × 250.

lies in cytoplasm which is barely tinted pink with no tendency toward localization. Only two cytoplasmic structures stand out sharply, even at low magnifications. These are (1) intensely stained small granules lying beneath the striated border and (2) the red to pink Golgi apparatus located between these and the nucleus. The Golgi apparatus appears as an irregular fine reticulum or network of red threads 0.3 micron or less in diameter, with thickenings along their extent which are about 0.6 micron or less in diameter. No clear "canals" are visible

in this region of the cell, nor are "chromophobic" masses detectable. In sections of the same material which have been stained by common procedures, the portion of the cell occupied by this structure appears as poorly defined clear areas, which constitute the "negative image." The Golgi apparatus stains more deeply in the columnar cells of the glands of Lieberkühn (figs. 2 and 3) than in those of the villus. In addition, the network is more compact and less reticulated in the former than in the latter (figs. 4 and 5). It is important to point out that mucigen granules of goblet cells are discrete spherical brilliant-red bodies, exceeding in color density any structure present in the columnar cells.

The behavior of the reactive components of the Golgi apparatus with reagents may be summarized as follows:

1. The staining of the Golgi apparatus is not markedly affected by buffers (p_H 3.6 to 8.0), dilute acetic acid (0.2 per cent), dilute hydrochloric acid (tenth normal) or many protein extractives.

2. After it has been treated with more concentrated solutions of acetic acid or with glacial acetic acid, the Golgi apparatus cannot be stained. It may be visualized if subsequently treated with hydrochloric acid and stained in the usual manner.

3. The Golgi apparatus is weakly stained after extraction has been carried on with dilute sodium or ammonium hydroxide or with ammonium sulfide.

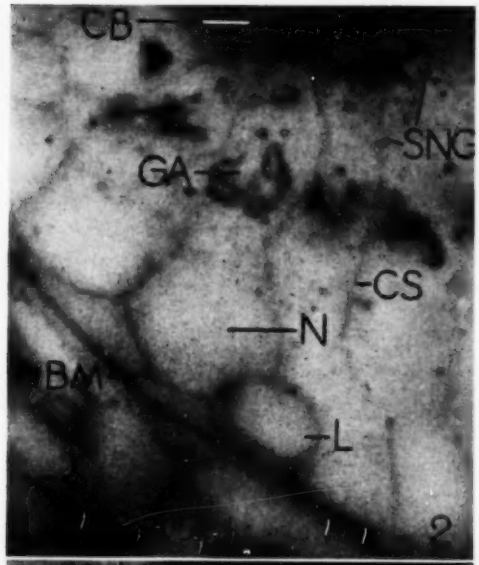
4. The Golgi apparatus cannot be stained after the action of pepsin, pangestin or trypsin.

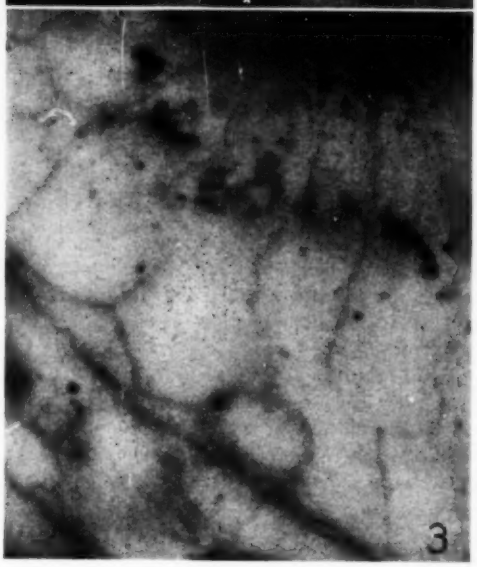
5. Although neither emulsin nor hyaluronidase has a notable effect on the intensity of staining of the Golgi apparatus, cytase and Clostridium welchii toxin cause marked decrease of its stainability.

COMMENT

The Golgi apparatus of columnar duodenal cells was described by Cajal,[5] Kopsch[6] and others after it had been visualized by the use of heavy metals. Morphologically, the apparatus which they described corresponds closely to the structure described in the foregoing pages. The chief difference is that with the use of heavy metals the constituent parts are somewhat coarser, possibly because of the effect of the deposition and apposition of reduced metal. Simpson's[7] describing of the Golgi apparatus in columnar cells fixed by freezing and drying as the vaguely defined "negative image" has been confirmed in the material used in this research. There seems little doubt that the structure I have described on the basis of the periodic acid—leukofuchsin method corresponds closely to, or is identical with, the structure which is commonly accepted as the Golgi apparatus.

5. Cajal, S. R.: Trab. d. Lab. de invest. biol. **3**:34, 1904.
6. Kopsch, F.: Ztschr. f. mikr.-anat. Forsch. **5**:221, 1926.
7. Simpson, W. L.: Anat. Rec. **80**:329, 1941.





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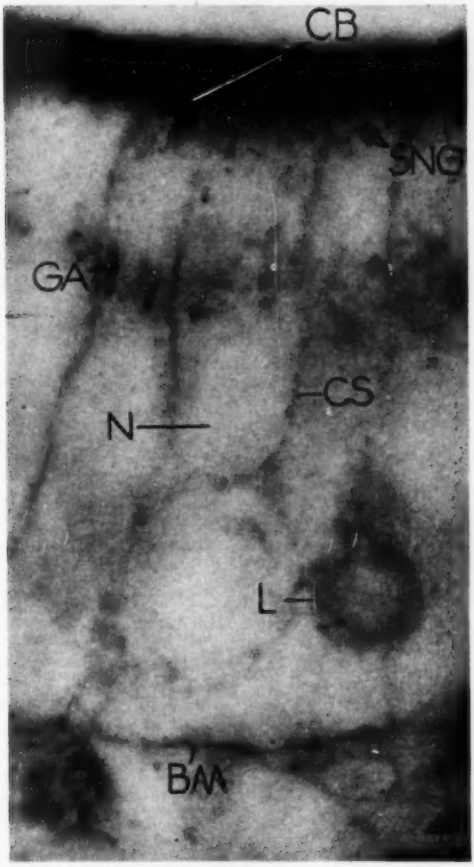

Fig. 4.—High power photomicrograph of surface epithelium of a villus prepared as described in the legends under the previous figures. In these cells the Golgi apparatus stains paler, and the structural details are finer, than in cells of the glands of Lieberkühn. The symbols are the same as those for figures 1 and 2. × 2,900.

Figs. 2 and 3.—High power photomicrographs of a portion of a transverse section of a duodenal gland of Lieberkühn of a rabbit after the tissue had been prepared by freezing and drying, sectioning and staining by the periodic acid—leukofuchsin method. The photomicrographs show the same cells taken at slightly different foci to show the delicacy of detail of the Golgi apparatus. *GA* indicates the Golgi apparatus; *SNG*, supranuclear granules; *CB*, the cuticular border; *N*, nucleus; *CS*, cement substance; *BM*, the basement membrane; *L*, a lymphocyte. × 2,900.

The reactive material present in the Golgi apparatus and responsible for visualization with the Hotchkiss method may be characterized, at least tentatively. Since there is no detectable decrement in the intensity

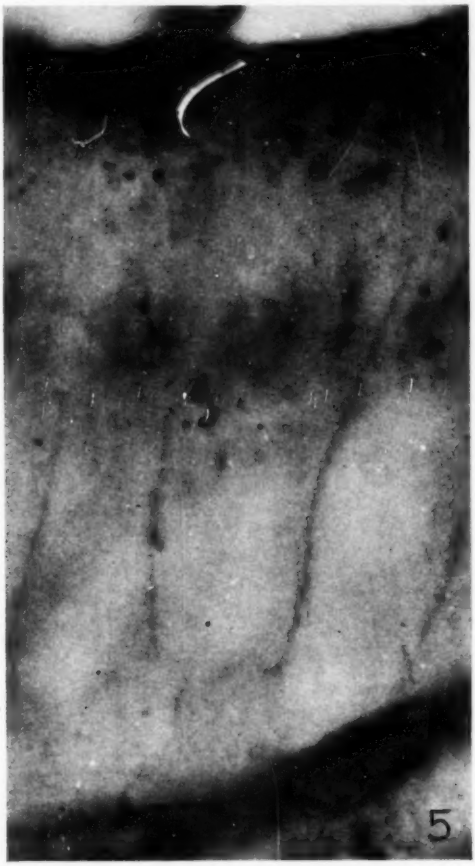

Fig. 5.—High power photomicrograph of surface epithelium of another villus taken at the same level as that in figure 4. × 2,900.

of the staining of the Golgi apparatus after the action of saliva and after extraction with a hot mixture of equal parts of methanol and chloroform, the reactive material may be said to be free of appreciable amounts of glycogen or lipid. This excludes the following classes of lipids (which

give positive results when spot-tested by the periodic acid—leukofuchsin method) as responsible for the colored reaction of the Golgi apparatus: kerasin, phrenosin, phosphatidyl ethanolamine, acetal phosphatide, inositol phosphatide, lecithin and ganglioside. Staining of the Golgi apparatus due to the presence of free aldehydes is excluded by the fact that it does not appear when the section is treated with the leukofuchsin reagent in the absence of prior oxidation by periodate. The reactive substance must then be a carbohydrate-containing protein or a lipoprotein. The distinction between these classes of proteins is not certain, but a number of lines of evidence suggest that the reactive substance of the Golgi apparatus may be a glycoprotein. These lines of evidence are: 1. Esterfied glycols fail to give a red color after oxidation with periodic acid and treatment with leukofuchsin. While it is unusual for acetic acid to form such compounds with simple carbohydrates, the negative reaction I have reported for the Golgi apparatus treated with strong acetic acid or acetic anhydride is understandable only on such a basis. The visualization of the Golgi apparatus following the hydrochloric acid treatment of such a negative section would appear, then, to be due to a reconversion of the altered groups to glycols, which are reactive. 2. The fact that snail stomach fluid and Cl. welchii toxin remove part of the reactive material of the Golgi apparatus clearly indicates the possibility that the reactive material may be a glycoprotein. The characteristic action of both of these preparations consists in the depolymerization of polysaccharides. In snail stomach fluid, the following enzymes have been identified: diastase, invertase, maltase, cellobiase, lipase and the mixture of enzymes known as cytase. Proteolytic enzymes are absent.[8] The following enzymes have been detected in the toxin of Cl. welchii: hyaluronidase, lecithinase, collagenase.[9] The marked reduction in the stainability of the Golgi apparatus following the action of these mixtures of enzymes indicates that the Golgi apparatus contains a complex polysaccharide. Together with the observations that the reactive groups are rapidly removed from the Golgi apparatus by pangestin, pancreatin and pepsin, the foregoing findings suggest that a carbohydrate-protein complex is the principal component of the structure visualized by the periodic acid—leukofuchsin technic. 3. It is a striking feature that alkaline buffers, dilute sodium or ammonium hydroxide, and ammonium sulfide reduce the stainability of the Golgi apparatus. These reagents also reduce the stainability of the mucigen granules of goblet cells. As these granules are considered to consist of glycoproteins, the findings suggest that the reactive groups of the Golgi apparatus contain similar components. 4.

8. Fabergé, A. C.: Stain Technol. **20**:1, 1945. Karrer, P.; Staub, M.; Weinhagen, A., and Joos, B.: Helvet. chim. acta **7**:144, 1924.

9. Oakley, C. L.; Warrack, G. H., and van Heyningen, W. E.: J. Path. & Bact. **58**:229, 1946.

Lipoproteins may possibly be present in the nucleus,[3] which is completely colorless after employment of the test procedure. This indicates that the lipid fraction (or the lipoprotein) of the Golgi apparatus, if present, is either nonreactive or is extracted by the procedure. As knowledge of the chemistry of the poorly characterized class of lipoproteins is still meager,[10] the possibility that some or all of the reactive groups of the Golgi apparatus may be parts of such a complex cannot be excluded at the present time. But, for the reasons cited, it would seem more profitable, at least tentatively, to regard the reactive components of the Golgi apparatus as alcohol groups of a polysaccharide-protein complex

Certain considerations follow if the Golgi apparatus is regarded as a structure containing polysaccharide. In both plants and animals, polysaccharides are frequently highly polymerized and possess properties of high viscosity or hardness, as, for example, cellulose, chitin, joint fluids, vitreous humor and the ground substance of the connective tissues. All of these structures may be considered ultimately as arising in part from the tendency of the constituent carbohydrate moieties to form submicroscopic structure through polymerization. It is possible that a similar tendency toward the formation of submicroscopic structure may be present in the Golgi apparatus. If this is so, it may be possible to conceive of the Golgi apparatus as a framework whose structure is of such a nature that it may accommodate certain enzymes or other activities in an orderly manner. Thus ascorbic acid[11] and phosphatase(s)[12] which have been described as lying in, or in the region of, the Golgi apparatus may be accommodated within this structure. Also lipid droplets, such as those described by Baker[1] in cells similar to columnar duodenal cells, may exist in relation to this structure, because lipogenetic enzymes may be attached to the glycoprotein structure. Other enzymes, which may be assumed to be involved in protein synthesis, may be attached in an orderly framework and give rise to the large number of presecretion droplets which are said to arise in the meshes of the Golgi apparatus. These speculations may be summarized in the following way: The Golgi apparatus may be a carbohydrate-protein complex, itself relatively inert, which possesses the peculiar submicroscopic structure that provides a suitable framework for the orderly arrangement of enzymes and other activities.

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10. Chargaff, E.: Lipoproteins, in Edsall, J. T., and Anson, M. L.: Advances in Protein Chemistry, New York, Academic Press, 1944, vol. 1.

11. Bourne, G.: Cytology and Cell Physiology, Oxford, Clarendon Press, 1942.

12. Emmel, V. M.: Anat. Rec. **95**:159, 1946. Dempsey, E. W., and Deane, H. W.: J. Cell. & Comp. Physiol. **27**:159, 1946; Deane, H. W., and Dempsey, E. W.: Anat. Rec. **93**:401, 1945.

Structures having the appearance of the Golgi apparatus have been observed, after employment of the periodic acid—leukofuchsin method, in fibroblasts in various sites and in glandular cells of the thyroid gland. Stainable bodies have also been observed in numerous other types of cells which, though localized in regions containing the Golgi apparatus, do not resemble it in structure. In still other cells no trace of stain has been observed in the region of the Golgi apparatus. This may mean that in different cells the Golgi apparatus contains little or no reactive components (possibly part of a glycoprotein) or that the hydroxyl groups of the polysaccharide exist not in a free or reactive state but rather in a substituted or nonreactive state. Such glycoproteins, if present, might be expected to possess submicroscopic structure and to play the same organizing role which has been postulated for the Golgi apparatus of the columnar cells of the duodenum.

SUMMARY

The periodic acid—leukofuchsin method was used to visualize the Golgi apparatus in columnar cells of the duodenum of the rabbit and the guinea pig after this tissue had been prepared by freezing and drying. The stainable component is classified, at least tentatively, as a carbohydrate-protein complex on the basis of the behavior of the reactive material with various reagents and enzyme preparations. The possibility that such a glycoprotein plays a role in cell metabolism is discussed.

ULCERS OF THE UPPER PART OF THE GASTROINTESTINAL TRACT ASSOCIATED WITH ACUTE DAMAGE OF THE BRAIN

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THE GENESIS of acute esophageal and gastroduodenal ulcers has long been a subject of clinical and experimental interest. As early as 1772, John Hunter[1] believed that the "dissolution of the stomach after death was due to post-mortem continuance of digestion." Hunter postulated a vital principle in all living tissue which prevented autodigestion. Claude Bernard[2] and his pupil Pavy cast doubt on this statement and showed that living tissue could be dissolved in gastric secretion. Recently Price and Lee,[3] by experimenting on gastric digestion in dogs, demonstrated that all living tissue underwent dissolution; in the hyperacid stomach all tissue, including the gastric mucosa, was dissolved more rapidly and more extensively.

Softening of esophagus and stomach in "acute affections of the brain or its membrane" was first described by Rokitansky[4] in 1849. He postulated that the lesion was probably due "to reflex action of the esophageal and gastric branches of the vagus, or to a diseased innervation of the stomach, owing to morbid condition of the vagus and to extreme acidification of the gastric juice." As Rokitansky's views have become known, clinicians, as well as experimental investigators, have forged a long chain of evidence connecting hemorrhages and ulcers of the stomach with cerebral lesions. Cushing[5] postulated the neurogenic origin of gastric ulcers and showed that lesions in the region of the third ventricle or the hypothalamus occasionally resulted in gastrointestinal ulceration. Cushing reported 11 cases in which ulcers and erosions of the esophagus and the stomach were associated with intracranial lesions.

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1. Hunter, J.: Philos. Tr. **62**:47, 1772.
2. Bernard, C.: Leçons de physiologie experimentale, Paris, 1855-1856.
3. Price, P. B., and Lee, T. F.: Surg., Gynec. & Obst. **83**:61, 1946; **84**:959, 1947.
4. Rokitansky, C.: A Manual of Pathologic Anatomy, London, New Sydenham Society, 1849, vol. 2, p. 35.
5. Cushing, H.: Surg., Gynec. & Obst. **55**:1, 1932.

Mastin and Bunts[6] presented 8 cases of neurogenic erosions and perforations of the stomach and the esophagus in support of Cushings' hypothesis. Boles and Riggs[7] presented 15 cases and brought forward the theory that the acute gastric erosions were due to circulatory insufficiency and had a common basis with the accepted peptic ulcer. They claimed that hypothalamic damage is not always a prerequisite but that increased intracranial pressure may be the necessary stimulus for the production of ulcer.

Fig. 1.—*A* (case 1), esophagus and stomach showing a perforation of the esophagus and four shallow ulcers and hemorrhagic erosions of the stomach. *B* (case 2), esophagus and stomach showing a perforation of the esophagus and ulcers and hemorrhagic erosions of the stomach. The two ulcers are at the pyloric end and the erosions are along the lesser curvature of the stomach.

In the 4 cases to be reported now, gastric ulceration occurred, and in 3 cases perforation of the stomach and the esophagus followed acute damage of the brain of diverse causation.

REPORT OF CASES

Case 1.—J. W., a man aged 63, was brought to the Toronto East General Hospital, Jan. 19, 1948, in an unconscious state, having been struck by an auto-

6. Mastin, M. G., and Bunts, R. P.: Arch. Int. Med. **54**:916, 1934.
7. Boles, R. S., and Riggs, H. E.: J.A.M.A. **115**:1771, 1940.

mobile shortly before. A swelling at the back of the head was noted. His breathing was heavy and stertorous. The left side of the face and the left arm were weak; the left leg was flaccid. Knee and ankle reflexes were absent. The pupils were small but equal and reacted sluggisly to light. The patient vomited rather abruptly a large amount of dark brownish fluid. A roentgenogram of the skull showed horizontal linear fractures of the right and left parietal bones.

On admission the patient was still unconscious, restless, moaning and tossing in bed and moving only the right side of his body. He vomited four times in the next twelve hours. The vomiting was projectile, and the vomitus was dark red. In the following two days, suction was utilized, and the return through the suction tube was dark red at all times. His temperature, pulse rate and respiratory rate remained around 102 F., 115 and 34, respectively, for the following three days. The spinal fluid was bloody, with a pressure of 235 mm. of water. The left side of his body remained weak. The patient never regained consciousness. He became progressively worse, with profuse sweating and difficult breathing. His temperature rose to 105 F., and death took place three and a half days after admission.

Autopsy, four hours later, revealed an extensive subdural hematoma over the right hemisphere, with laceration and edema of the temporal lobes and the lower part of the frontal lobe. Hypothalamic hemorrhages were noted. There were linear fractures of the left temporal bone extending downward and forward to the basisphenoid and the outer table of the right parietal bone. In the pyloric and lesser curvature regions of the stomach there were four shallow ulcers, each about 1.5 cm. in diameter, arranged in a crescentic pattern. The wall of the stomach was greatly dilated. There was a scattering of hemorrhagic erosions in the lower portions of the gastric mucosa. The esophagus had lost its normal mucosal markings and showed a grayish black linear rent measuring 4 by 2 cm. and communicating with the mediastinum. This linear rent extended into the cardiac end of the stomach. One hundred cubic centimeters of dark brown gastric contents was found in the posterior mediastinum. This fluid gave a reading of 52 units of free hydrochloric acid. There was marked edema of both lungs, with early bronchopneumonia of the right lung. The pleura of the lower lobe of the right lung was adherent to the diaphragm and the mediastinum.

Histologic examination of the esophagus revealed necrosis and edema of all layers at the edge of the perforation with moderate mobilization of polymorphonuclear and mononuclear leukocytes. The mucosa was denuded, with congestion and hemorrhage of the adjacent tissue. In the erosions of the stomach, the centers of the craters extended down to the submucosa, with sloping edges. The surrounding zone showed edema, congestion, hemorrhage and infiltration with lymphocytes and a minimal number of polymorphonuclear leukocytes.

Microscopic examination of the hypothalamus showed marked congestion, numerous ring hemorrhages and pericellular edema.

Case 2.—L. W., a white woman aged 40, was brought to the Toronto East General Hospital, Jan. 21, 1948, two hours after a sudden collapse at home. She had been perfectly well until 7:30 a.m., when she dropped to the floor unconscious.

The patient was deeply unconscious, not responding to any stimuli. Her temperature, pulse rate and respiratory rate were 98.6 F., 70 and 24, respectively. Blood pressure was 90 mm. systolic and 70 mm. diastolic. Neurologic findings were normal except for two small hemorrhagic spots on the left disk. The spinal fluid was under pressure, with a hemoglobin reading of 5 per cent, a red blood cell count of 400,000 and a leukocyte count of 600 cells per cubic millimeter. The

patient vomited four times the first day and occasionally during the next few days The hemoglobin content of the blood was 89 per cent; the red blood cell count was 4,500,000 and the white cell count 9,100 per cubic millimeter. The Kahn test of the blood was positive up to 240 units. The urine indicated moderate proteinuria and showed granular casts. The blood nonprotein nitrogen was 31 mg. per hundred cubic centimeters.

This patient was incontinent of urine and feces within four hours after admission. Later she roused slightly from her coma, opened her eyes and moved her right arm first, with slow movement of all limbs following, and responded to moderate stimuli. In the following four days she was extremely restless; the mental state alternated between light sleep, from which she was easily aroused, and wakefulness, when she tossed and struggled.

January 23 the patient had a cough and signs of bronchopneumonia in the lower half of each lung posteriorly. Her temperature went up to 102.8 F., swinging between 100 and 104 F.; the pulse rate was 135 to 80; the respiratory rate, 60 to 30. On January 26 her temperature rose to 106 F. Bilateral choking of the disks, bilateral up-going of toes and marked rigidity of the neck were noted. On the last day the spinal fluid was serous pink, with a red blood cell count of 12,000 and a white cell count of 20 per cubic millimeter. Smear and culture revealed no micro-organisms. The patient died at 12:30 a.m., five days after admission.

Autopsy, six hours later, revealed an area of fresh circumscribed hemorrhage, 2 cm. in diameter, in the island of Reil on the right side. There was a small softening through the cortex, communicating externally with the subarachnoid space, and there was basal pooling of blood. There was bilateral basal bronchopneumonia with hydrothorax—200 cc. of blood-tinged fluid in the left thoracic cavity and coffee-colored fluid in the right—and gastric contents were found in the mediastinum. A perforation in the cardiac end of the esophagus about 6 cm. by 2 cm. was found. The lower third of the esophagus was soft, partially shredded and slate colored. The stomach was dilated and there were two superficial ulcers, each about 1 cm. in diameter, at the pylorus and small hemorrhagic erosions along the lesser curvature.

The cortex at the island of Reil showed extensive hemorrhage. Throughout the leptomeninges there were congestion, moderate perivascular lymphocytic infiltration and early endarteritis. The inflammatory response to the ulcers of the fundus of the stomach and to the esophageal perforation was minimal. A few leukocytes occupied perivascular sites at the margins of the gastric ulcers. The lymphocytic and mononuclear response was greater in the region of the esophageal slough.

Case 3.—F. W., a woman aged 74, was admitted unconscious to a city hospital on March 5, 1948, following a car accident. Her pupils did not react to light. The reflexes were all present. There were lacerations of the nose and over the occipital area and a fracture of the upper thirds of the right tibia and fibula. The patient remained semiconscious; her temperature ranged from 99.2 F. on admission to 105 F. on March 9 and 10. Her condition steadily declined, and she died five days after the accident.

Autopsy, fifteen hours after death, revealed: subdural hemorrhage over the left superoinferior temporal region and deep cerebral laceration and softening of the left suboccipital area, communicating with the lateral ventricle; fracture of the upper thirds of the right tibia and fibula; basal pneumonia; hyperostosis frontalis interna; pedunculated submucosal lipoma of the pylorus; follicular cysts of the ovaries, and two acute duodenal ulcers, one measuring 2 by 1 cm. and the other 0.5 cm. in diameter. The esophagus, the stomach and the small and large

bowel otherwise were normal in appearance. There was an organized traumatic hemorrhage replacing the right adrenal gland. Tarry stools were noted in the colon.

The brain revealed edema of the left hemisphere. There were, in addition, a cortical hemorrhage 1 cm. in depth in the anterior pole of the left frontal lobe and a deep laceration 5.5 by 3 cm. opening into the third ventricle. This laceration involved the left inferior temporal gyrus, the fusiform gyrus and part of the hippocampal gyrus. There was no gross destruction in the hypothalamic region.

Microscopic examination of the superficial ulcers showed excavation down to the muscularis, and the margins were steeep. On the floor of the craters there was a zone of desquamated epithelium and cellular debris and an underlying early fibrinoid change. In the adjacent tissue around the ulcers there was a heavy infiltrate of polymorphonuclear leukocytes.

Case 4.—A 12 year old boy was admitted, March 27, 1948, to Toronto East General Hospital for tonsillectomy. He was anesthetized with ethyl chloride followed by ether. During the operation, there was apparently considerable difficulty in maintaining an adequate airway, and the patient became cyanosed at times. At the end of the operation, he suddently ceased breathing and became deeply cyanosed and the heart stopped beating. Cardiac massage was performed immediately through a left oblique subcostal incision, and at the same time intubation was carried out and artificial respiration started. The heart began to beat again four minutes later, and spontaneous respiration returned. Postoperatively the patient was deeply unconscious, with intermittent muscular twitching and clonic convulsions. He died thirty hours after the accident. His temperature remained around 104 to 106 F.; the pulse rate varied between 110 and 160 per minute and the respiratory rate between 30 and 44 per minute during this period of thirty hours of second life.

Autopsy, two hours and fifteen minutes later, revealed marked edema of the brain with a pronounced tentorial and cerebellar cone of pressure. Also discovered were malacia and multiple perforations of the stomach, an acute ulcer of the duodenum and in the left subphrenic space 200 cc. of gastric contents with free hydrochloric acid estimated as 16 units, giving a total of 94 units. Other findings were petechiae of the pericardium, the lungs and the thymus.

The stomach showed a dilated fundus. The entire wall except part of the antrum was thin. Multiple erosions and perforations were present. There were five perforations of varying size. Three were located along the greater curvature—one on the anterior surface, 2 by 1 cm., and two on the posterior surface, 3.5 by 1.2 cm. and 0.4 cm. in diameter, respectively. Two were located along the lesser curvature, one measuring 1 by 0.3 cm. and the other 0.2 cm. in diameter. The mucous membrane appeared white and greenish gray shreds were adherent to the wall. A few hemorrhagic spots were present over a portion of the pylorus.

Histologic examination of the stomach near the perforation showed digestion and necrosis of all layers. The submucosa was markedly edematous. No cellular infiltration was found. Section of the duodenal ulcer showed that the crater extended down to the muscularis. Focal areas of hemorrhagic erosion surrounded by polymorphonuclear leukocytes were found in the adjacent mucosa. There was extensive edema in the submucosa, with heavy polymorphonuclear infiltration down to the muscularis mucosae.

Tissue from the cortex, the hypothalamus and the basal ganglions showed marked congestion and red cell stasis in the capillaries and larger vessels. The endothelium of a few vessels revealed early degenerative changes with disap-

pearance of cellular structure, while other vascular channels showed mural swelling with prominent large pale cells and nuclei. There was marked distention of the perivascular spaces, which gave a sievelike appearance. The nerve cells were swollen, with distortion of nuclei. The cytoplasm showed liquefacient change with partial to complete loss of tigroid substance. Most of the nerve cells of the cortex were completely degenerated, leaving only their outlines. The nuclei of the oligodendrocytes were moderately swollen and pale. Acute swelling of these cells was well advanced. The astrocytes were swollen and vesicular. The microglia cells were free from nuclear transformation.

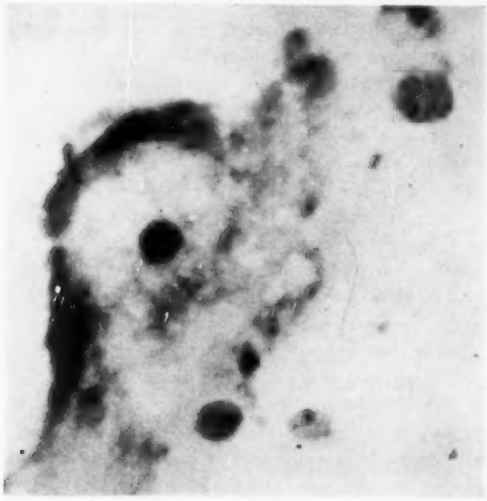

Fig. 2 (case 4).—Photomicrograph of a neuron of the hypothalamus showing degeneration, with pyknosis of the nucleus, peripheral condensation of tigroid substance and liquefacient change of the cytoplasm. Nissl stain; × 1,640.

COMMENT

Erosions and ulcerations of the upper part of the gastrointestinal tract were seen at autopsy in 4 cases of acute craniocerebral damage. These were the only cases in which such lesions were found out of 210 cases of acute fatal head injuries in which autopsy was performed by one of us (Dr. Wyatt) in a study of head injuries in the south of England during the last war and more recently as a coroner's pathologist. The major portion of the 210 patients did not survive for a sufficient length of time for gastrointestinal ulcers to develop.

All 4 patients with "neurogenic ulcers" were unconscious from the onset and lived between one and a half and five days. Nutrition was sustained with 5 per cent dextrose in saline solution and other supplements

administered by intravenous drip. No food was taken by mouth; hence no active digestion of food took place before or at the time of death. The possibility of postmortem digestion of a hyperacid stomach or continuance of digestion in an agonal state was minimized. Two patients who were free of nasopharyngeal bleeding gave evidence of hemorrhage of the upper gastrointestinal tract by vomiting dark bloody gastric contents a few hours after admission. One patient had tarry stools. All of the patients were unconscious, and hence could not feel the pain of perforation. In cases 1 and 2 vomiting with its definite traumatic effect on the esophagus could not be clearly assessed although it is well known that vomiting plays a definite role, as in the Mallory-Weiss[8] syndrome.

In the care of patients with acute cerebral damage there are several clinical features of importance—for instance, the development of an acute pathologic condition of the abdomen, the vomiting of blood or the passage of tarry stools. These features should arouse interest and thought as to the presence of a so-called neurogenic ulcer.

Microscopically, destruction of tissue associated with congestion and thrombosis of submucosal vessels and a variable amount of lymphocytic and polymorphonuclear leukocyte infiltration at the edge of scattered ulcers were found in all 4 cases. The finding of this inflammatory infiltrate was evidence for the antemortem existence of these ulcers.

It is interesting that around the gastric ulcers cellular reaction was minimal to moderate in cases 1 and 2 and absent in case 4, while around the duodenal ulcers there was an extensive concentration of polymorphonuclear leukocytes in cases 3 and 4. The question arises why there was less cellular response to acute gastric ulcers than to duodenal ulcers if they developed at the same time. Morphologically, the duodenal ulcers were of a very early nature.

Several hypotheses can be brought forward: The presence of certain physiologic factors, such as the dissolutive action of the acid gastric juice, may have hastened digestion at the sites of hemorrhagic erosion in the gastric mucosa, with perforation following rapidly before any mobilization of leukocytes was possible; or a chemical factor, such as the acid hydrogen ion concentration of the eroded gastric mucosa, may have exerted an influence such that the mononuclears were left dominant over the polymorphonuclears in the surrounding tissues, or tissue leukocytes may have been inhibited.

The sites of the ulcers of the stomach and the esophagus were examined for evidence of heterotopic alkaline mucosa. No displacement of mucosa was found in any of the cases.

One theory previously propounded was that in unconscious patients there is a resultant atony of the cardiac sphincter allowing the acid gas-

8. Mallory, G. K., and Weiss, S.: Am. J. M. Sc. **178**:506, 1929.

tric juice to be regurgitated into the esophagus and aiding postmortem digestion. The observation that inflammatory cells, an antemortem stigma of inflammation, were present must be regarded as frank evidence against the postmortem malacia theories in these cases.

Fundamental damage of the brain was common to all the cases, although it varied in origin: in 2 it was of traumatic genesis; in 1 it was due to a spontaneous intracranial hemorrhage with basal pooling of blood and was linked with positive serologic tests indicating syphilis; the fourth case was an example of damage due to anesthetic anoxemia.

Since the time of Cushing the casual relationship of hypothalamic injury and gastric erosions seems to have been well established by experimental workers (Keller, Hare and d'Amour[9]; Watt and Fulton[10]; Hoff and Sheehan[11]), but the exact mechanism of the formation of ulcers is still in dispute. One school (Watt and Fulton[10]; Hoff and Sheehan[11]) has suggested that the gastrointestinal changes are the result of local gastric ischemia due to hyperactivity of the sympathetic vasoconstrictive mechanism. Another school (Cushing[5] and Beattie[12]) advanced the thought that the gastric erosions and hemorrhage are due to stimulation of the parasympathetic center through its efferent fibers contained in the vagus nerve; this nerve conducts the impulse to the gastroduodenal musculature resulting in spasmodic contraction producing ischemia and hemorrhagic erosion leading to ulcer formation. The third school (Boles and Riggs[7]; Penner and Bernheim[13]) expressed the belief that the vasomotor mechanism stimulates peripheral circulatory stasis, leading to ulceration of the gastrointestinal tract. This ulceration results either from central vegetative stimulation through intracranial lesions, or is due to a stimulation of the cranial vegetative center resulting from circulatory failure. These authors also brought forward the thought that systemic disease initially would produce general peripheral stasis with prolonged vasoconstriction and resultant development of acute gastrointestinal ulcers.

These hypotheses are not in conflict fundamentally, as ulceration and hemorrhage have been reported in animals after posterior pituitary injection U.S.P. (pituitrin$^{(R)}$) and pilocarpine hydrochloride had been injected into the lateral ventricles (Cushing[14]; Light Bishop and Ken-

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10. Watt, J. W., and Fulton, J. J.: Ann. Surg. **101**:363, 1935.

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12. Beattie, J.: Canad. M. A. J. **26**:278, 1932.

13. Penner, A., and Bernheim, A.: Arch. Path. **28**:129, 1939.

14. Cushing, H.: Proc. Nat. Acad. Sc. **17**:163 and 239, 1931.

dall[15]), after acetylcholine and betahypophamine (pitressin(R)) had been injected intravenously (Necheles and Masur[16]) and after epinephrine hydrochloride had been injected intraperitoneally (Penner and Bernheim[17]). Moreover, Keller,[18] studying the relation of the peripheral nerves and gastric ulcer, observed that bilateral vagotomy performed previous to injury of the hypothalamus resulted in a typical hemorrhage without formation of a crater, while bilateral thoracic and abdominal sympathectomy carried out before injury of the hypothalamus produced erosions without hemorrhage. From all these observations it seems that both parasympathetic and sympathetic nerves are concerned in the causation of gastrointestinal ulceration and hemorrhage. The present concepts are more in favor of sympathetic overstimulation. It is of interest to mention that the bilateral vagotomy[19] advocated for peptic ulcer nowadays has gained some popularity, which further emphasizes the paramount role played by the central nervous system in the production of ulcer.

SUMMARY

Four cases in which "neurogenic ulcer" occurred with varied types of brain injury are reported. In 4 cases criteria of antemortem inflammation were presented. The pathogenesis and experimental contributions bearing on it are reviewed. From the point of view of care and treatment, awareness of the condition is important in all cases of acute cerebral injury.

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DIET AND CIRRHOSIS OF THE LIVER

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BY THE needle biopsy technic (Wahi[1]) it has been possible to study histologically livers of a large number of patients who were admitted to the Thomason Hospital, Agra, for varied symptom complexes. During the study I have been impressed with the frequency of hepatic injury of varying grades in patients not primarily admitted for disease of the liver. Cirrhosis of the liver is common among Indians, and the patients with this disease are usually drawn from two classes of society, orthodox Hindus, who are strict vegetarians, and the poorer classes, who eat what they grow or what is cheapest. The diets of both these sections of the population are ill balanced.

Nutritional disease of the liver can be produced in animals by a variety of means and the resulting lesions have attracted great attention in recent years. Although some workers have been overenthusiastic in applying what they have observed in animals to the diseases of human beings, any relationship between the experimentally produced disease of the liver and that occurring in man is far from established.

REVIEW OF THE LITERATURE

That diet influences the degree to which the liver is sensitive to injury is no new discovery. Opie and Alford,[2] in 1914, and Davis and Whipple,[3] in 1919, showed that the degree to which their animals were susceptible to chloroform poisoning depended largely on the character of the diet they were receiving. In the years following, the work was directed largely toward determining the influence of diet on the hepatic injury caused by exposure to definite hepatic poisons. Weichselbaum[4] in 1935 was the first to demonstrate that dietary factors alone can initiate injury of the liver. He noticed that a dietary deficiency of protein, par-

From the Laboratory of Pathology, New England Deaconess Hospital, Boston, and the Department of Pathology, Medical College, Agra, India.

1. Wahi, P. N.: Indian M. Gaz. **81**:130, 1946.
2. Opie, E. L., and Alford, L. B.: J.A.M.A. **62**:895, 1914.
3. Davis, N. C., and Whipple, G. H.: Arch. Int. Med. **23**:612, 1919.
4. Weichselbaum, T. M.: Quart. J. Exper. Physiol. **25**:363, 1935.

ticularly of sulfur-containing amino acids, caused death with hemorrhage of the liver. Since then, hepatic injury resulting from dietary deficiencies has attracted increasing attention, and new information concerning the causation of hepatic disease has been acquired. It may therefore be opportune to review and reorient present concepts of hepatic damage in the light of the experimental findings.

EXPERIMENTAL PRODUCTION OF LESIONS IN THE LIVERS OF ANIMALS BY DEFICIENT DIETS

Diets Deficient in Proteins.—Hepatic lesions produced by dietary deficiency did not attain prominence until 1940, when Rich and Hamilton[5] reported that cirrhosis of the liver had been produced in rabbits by a synthetic diet lacking in yeast but supplemented by vitamins. The same authors[6] described cirrhosis of the liver resembling Laennec's cirrhosis of man occurring in all of 14 rabbits kept from 25 to 113 days on diets lacking yeast but supplemented by various vitamins. In some of the animals ascites also developed. They concluded that cirrhosis was due to the lack of some factors of yeast other than thiamine, riboflavin, pyridoxine and nictotinic acid. Probably it was choline. These findings became all the more interesting in view of the observations of Von Glahn and Flinn[7] that yeast added to the diet of rabbits reduces somewhat the tendency of liver to be damaged by lead arsenate.

Lillie, Daft and Sebrell[8] produced cirrhosis of the liver in rats with a diet which was low in protein (4 to 10 per cent) and in sulfur-containing amino acids but which was supplemented with thiamine, nicotinic acid, pyridoxine and pantothenic acid. The fat content of this diet varied from 5 to 70 per cent. They found that on the average the pathologic changes were more severe when approximately 20 per cent alcohol was substituted for drinking water. Lowry and co-workers[9] reported that treating with choline or increasing the casein content of the diet to 50 per cent resulted in hyperplastic regeneration of the liver and clinical improvement of rats with experimentally produced cirrhosis of the liver. In 1942 Lillie and associates,[10] describing the histologic aspects and the

5. Rich, A. R., and Hamilton, J. D.: Tr. A. Am. Physicians **55**:133, 1940.

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8. Lillie, R. D.; Daft, F. S., and Sebrell, W. H.: Pub. Health Rep. **56**:1255, 1941.

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10. Lillie, R. D.; Ashburn, L. L.; Sebrell, W. H.; Daft, F. S., and Lowry, J. V.: Pub. Health Rep. **57**:502, 1942.

pathogenesis of cirrhosis of the liver, stated that among the noteworthy features is the absence of hemorrhage and, in the earlier stages, obvious necrosis of liver cells.

Gillman and co-workers[11] and Gillman and Gillman[12] found, from studies of biopsy specimens of liver, that extensive damage of the liver is invariably present in cases of malnutrition, including pellagra and nutritional edema, whether the patients are children or adults. Gilbert and associates,[13] Gillman,[14] Gilbert and Gillman[15] and Gillman and co-workers[16] showed that severe damage of the liver develops in young rats fed ad libitum on a diet of mealie pap (maize meal porridge) and sour milk. It expresses itself as diffuse fatty liver, cirrhosis, lobar absorption or diffuse lobar enlargement. One or more of these conditions may be present in the same liver. Mealie pap, supplemented frequently by fermented whole cow's milk, is the staple diet of many South African Negroes; it has a high carbohydrate and low protein content.

Himsworth and Glynn[17] reported that massive hepatic necrosis was produced in rats by low protein diets. They found that proteins vary in their power to prevent the lesion, indicating thereby that the protective factor is a component of protein rather than the intact protein molecule itself. They[18] further found that massive hepatic necrosis due to a protein-deficient diet is prevented in rats by adding either casein (8 per cent) or an equivalent amount of dl-methionine to the diet. The lesion was not prevented by l-cystine in an amount equivalent to that contained in an 8 per cent casein diet. A daily supplement of 4 mg. of choline to each animal or a combination of choline and cystine in that amount was equally ineffective. They came to the conclusion that the protective action of casein is due to the methionine contained in it. They found no evidence that hepatic necrosis results from vitamin or mineral deficiency.

Fouts[19] reported the development of a deficiency state characterized by loss of weight, moderate anemia, dermal and peptic ulcers, a fatty,

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17. Himsworth, H. P., and Glynn, L. E.: (*a*) Clin. Sc. **4**:421, 1942; (*b*) **5**:93, 1944; (*c*) Lancet **1**:457, 1944.

18. Himsworth, H. P., and Glynn, L. E.: Clin. Sc. **5**:133, 1944.

19. Fouts, P. L.: J. Nutrition **25**:217, 1943.

cirrhotic liver and death in dogs fed a diet low in protein (15 per cent casein) but supplemented with thiamine, riboflavin, nicotinic acid, pyridoxine and pantothenic acid. An increase in the amount of protein (41 per cent) prevented the condition.

Li and Freeman[20] reported that fatty liver developed in dogs maintained on a diet that was 33 per cent fat and deficient in protein. Waterlow,[21] discussing the etiologic factors of the so-called "fatty liver disease" of West Indian infants, pointed out that all the evidence, including the history, the associated lesions and the response to treatment, suggested that the underlying deficiency was either in protein or in some member of the vitmain B complex.

Diets Deficient or Ill Balanced in Amino Acids.—Since the recognition of fatty infiltration of the liver as a definite precirrhotic condition, the lipotropic activity of various amino acids has been a subject of intense investigation. The existence of lipotropic factors was first indicated by Hershey[22] and Hershey and Soskin,[23] while they were studying the development of fatty liver in pancreatectomized dogs maintained on insulin. Accepting the view that phospholipids were essential for fat transport, Hershey[22] fed his animals lecithin and noted marked reduction of liver fat. Best, Hershey and Huntsman[24] and Best and Huntsman[25] showed that addition of choline, a constituent of phospholipids, brought the livers of their rats to normal. Both choline and lecithin were found to prevent the accumulation of fat and to effect its mobilization from an already infiltrated organ. These results have been corroborated by Best and Ridout[26] and Best, Channon and Ridout,[27] who demonstrated a similar effect for cholesterol deposited in the liver.

The lipotropic activity of various proteins has since been investigated. Besides lecithin and choline, casein was found to be lipotropic, exerting its maximum effect when it constituted 30 per cent of the diet (Best and Huntsman[28]; Channon and Wilkinson[29]). The list of proteins exerting lipotropic action has now been extended to include egg white and beef

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27. Best, C. H.; Channon, H. J., and Ridout, J. H.: J. Physiol. **81**:409, 1934.

28. Best, C. H., and Huntsman, M. E.: J. Physiol. **83**:255, 1935.

29. Channon, H. J., and Wilkinson, H.: Biochem. J. **29**:350, 1935.

muscle powder (Best, Grant and Ridout[30]) and edestin, fibrin and gliadin (Channon and co-workers[31]). On the other hand, lysine, glutamic and aspartic acids, glycine, alinine, valine, serine, phenylalanine, proline, hydroxyproline, leucine, histidine, arginine and cystine have no lipotropic action (Beeston and Channon;[32] Beeston, Channon and Platt;[33] Beeston and Platt[34]). Cystine has been found to be alipotropic; its use leads to further accumulation of fat in the livers of animals fed a fatty diet. The studies of György, Poling and Goldblatt,[35] Blumberg and McCollum[36] and Earle and Victor[37] showed that extra cystine added to the experimental diets accelerated the development of cirrhosis.

Tucker and Eckstein[38] suggested that the lipotropic activity of a protein was due to the opposing effects of the cystine and the methionine contained in it. The lipotropic activity of methionine was confirmed by Channon, Platt and Smith,[39] Best and Ridout[40] and Best and Lucas.[43] The effect of protein on fat transport has been satisfactorily explained as being due to the supply of methyl groups for the synthesis of choline (McHenry and Patterson[42]). This has been further proved by studies of the metabolism of methionine in which labeled elements, like heavy hydrogen and radioactive sulfur, were used, which showed that the lipotropic action of methionine is associated with its methyl group. This is transferred intact in the synthesis, within the body, of such methyl compounds as creatine, sarcosine and choline (DuVigneaud and associates[43]). Griffith[44] showed that in young rats fed a diet low in

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44. Griffith, W. H.: J. Nutrition **21**:291, 1941.

choline hemorrhagic degeneration was absent or slight if the diet contained more than 0.9 per cent of methionine, regardless of whether this level was attained by administering supplementary methionine or by insuring protein high in casein, lactalbumin or fibrin. Thus choline is a dietary essential if the ration contains less than 0.8 per cent methionine. Choline requirements vary inversely with dietary methionine and are increased by dietary cystine.

The alipotropic action of cystine was investigated by Beeston and Channon,[32] who drew attention to an observation of Curtis and Newburgh[45] that when cystine constituted 0.75 to 20 per cent of the diet it produced in addition to necrosis a large increase of fat. There is no agreement on the manner in which cystine exerts its alipotropic effect. Best and Lucas[41] thought that this action was probably nonspecific and due to the raising of metabolism nearer to normal, so that there was increased demand for lipotropic factors. On the other hand, Treadwell and co-workers[46] indicated the possibility that there was direct antagonism between methionine and cystine. An excess of fat accumulated in the liver is an evidence of an alteration of metabolism due either to failure of the transport of hepatic fat or to a withdrawing of fat from body stores so rapidly that the liver is unable to cope with the fat brought to it. Mulford and Griffith[47] showed that in a diet containing 18 per cent casein the sulfur level is suboptimal for the growth of rats (0.14 instead of 0.19 per cent) and that a supplement of sulfur (0.05 per cent) as cystine causes increased growth without corresponding increased intake of food. They suggested that in this process methionine is diverted to form tissue proteins and is thus deprived of its lipotropic action. For a detailed discussion of the mode of action of lipotropic and alipotropic factor the reader is referred to an excellent review by Glynn.[48]

Diets Rich in Fats.—Besides protein deficiency, diets rich in fats have been reported to cause damage of the liver. Chaikoff and associates[49] demonstrated that cirrhosis of the liver occurs in completely depancreatectomized dogs. A constant finding in the liver was an early increase of the hepatic fat. The sequence of events was fatty infiltration, hyaline degeneration, atrophy of the cells at the periphery of the lobule, and fibroblastic proliferation ending in a typical hobnail liver. In such animals fatty infiltration of the liver is probably due to the absence of

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a pancreatic enzyme which enables methionine to act as a lipotropic factor (Chaikoff, Entenman and Montgomery[50]).

Blumberg and Grady[51] observed cirrhosis of the liver of the diffuse nodular type in rats fed large quantities of wheat germ oil or corn oil for approximately 200 to 400 days. A large oil supplement was added to the basal ration so that the protein content was reduced to about 10 per cent and the fat increased to about 50 per cent. This resulted in prolonged fatty infiltration followed by cirrhosis. The addition of choline prevented the production of cirrhosis.

That fatty infiltration of the liver is an important precursor of cirrhosis of the liver has been shown by many workers. Cholesterol has long been known to increase the lipid content of the liver (Best and Ridout[52]). Cirrhosis has been observed in rabbits after the administration of cholesterol by Chalatow[53] and more recently by Leary.[54] Connor[55] showed that long-continued fatty infiltration of the liver is a factor of great importance in the development of cirrhosis in diabetes and chronic alcoholism. In both conditions he attributed it to an alteration of the metabolism of the hepatic carbohydrate and fat. Blumberg and McCollum[36] reported that cirrhosis of the liver developed in more than 300 rats fed a high fat (55 to 70 per cent) and low protein (10 per cent casein) diet. By the addition of choline they prevented its development in a like number of animals.

Webster[56] and Handler and Dubin[57] described necrosis and cirrhosis of the liver in rats receiving diets poor in protein and choline but rich in fat. The hepatic lesions were prevented by increasing the protein content of the diet (casein from 8 to 18 per cent) and by the addition of molasses. A reduction in the fat content or the addition of betaine diminished the severity of the lesion, while the addition of cystine and cholesterol increased it.

Himsworth and Glynn[18] reported that diffuse hepatic fibrosis (portal cirrhosis) was produced by diets containing an excess of fat (51 per cent) and leading to long-continued fatty infiltration of the liver. Since the

50. Chaikoff, I. L.; Entenman, C., and Montgomery, M. L.: J. Biol. Chem. **160**:489, 1945.

51. Blumberg, H., and Grady, H. G.: Arch. Path. **34**:1035, 1942.

52. Best, C. H., and Ridout, J. H., in Luck, J. M.: Annual Review of Biochemistry, Stanford University, Calif., Annual Reviews, Inc., 1939, vol. 8, p. 349.

53. Chalatow, S. S.: Beitr. z. path. Anat. u. z. allg. Path. **57**:85, 1914.

54. Leary, T.: Arch. Path. **32**:507, 1941.

55. Connor, C. L.: (*a*) Am. J. Path. **14**:347, 1938; (*b*) J.A.M.A. **112**: 387, 1939.

56. Webster, G. T.: J. Clin. Investigation **21**:385, 1942.

57. Handler, P., and Dubin, I. N.: J. Nutrition **31**:141, 1946.

diffuse hepatic fibrosis depended on the preceding prolonged fatty infiltration, a diet low in lipotropic factors had the same effect as the one rich in fat.

Diets Deficient in Vitamins.—Vitamin deficiencies have also been charged with the production of hepatic injury. György and Goldblatt[58] observed various pathologic changes in 48 rats kept on a basal diet deficient in the vitamin B complex but supplemented with thiamine and riboflavin or with thiamine, riboflavin and pyridoxine. These changes were characterized by parenchymatous and fatty degeneration, focal and massive necrosis, hyperemia and hemorrhage and, in some rats, by perilobar and condensation fibrosis. The addition of yeast or a yeast extract (Peter's[58a] eluate) prevented the hepatic injury, while the condition was aggravated by l-cystine. They came to the conclusion that the hepatic changes were dependent on a deficiency of a part of the "vitamin B_2 complex." Later the same authors[59] reported that it was deficiency of choline in rats kept on the basal ration commonly used in the study of the "vitamin B_2 complex" which was responsible for the production of fatty infiltration of the liver. They could prevent fatty infiltration by adding 2 mg. of choline per gram of the experimental diet or by the addition of dl-methionine.

It is an accepted view that certain of the "B vitamins" influence the fat content of the liver. McHenry[60] reported that thiamine encouraged the development of fatty liver in choline deficiency, probably by converting carbohydrate to fat. Halliday[61] attributed lipotropic activity to pyridoxine (B_6), but this was disputed by Gavin and McHenry.[62] Their failure to produce fatty liver with pyridoxine-deficient diets may be due to the shorter periods of their feeding (Engel[63]). Biotin, on the other hand, causes hepatic deposition of fat (McHenry and Gavin[64]), an effect which is inhibited by inositol but not by choline (Gavin and Mc-

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58. György, P., and Goldblatt, H.: J. Exper. Med. **70**:185, 1939.

58a. Kinnersly, H. W.; O'Brien, J. R. P.; Peters, R. A., and Reader, V.: Biochem. J. **27**:225, 1933.

59. György, P., and Goldblatt, H.: (*a*) J. Exper. Med. **72**:1, 1940; (*b*) Proc. Soc. Exper. Biol. & Med. **46**:492, 1941; (*c*) J. Exper. Med. **75**:355, 1942.

60. McHenry, E. W.: J. Physiol. **86**:27, 1936; Science **86**:200, 1937.

61. Halliday, N.: J. Nutrition **16**:285, 1938.

62. Gavin, G., and McHenry, E. W.: J. Biol. Chem. **132**:41, 1940.

63. Engel, R. W.: J. Nutrition **24**:175, 1942.

64. McHenry, E. W., and Gavin, G.: Proc. Am. Soc. Biol. Chem. **35**: lxxxvii, 1941.

Henry[65]). Best and co-workers[66] confirmed the lipotropic action of inositol but failed to find any evidence of an action inhibitory of biotin.

It is probable that both fatty infiltration and vitamin deficiency play a role in the development of cirrhosis of the liver. (Greene[67]). Spellberg and Keeton[68] produced fatty degeneration of the liver in guinea pigs and rabbits with diets lacking in vitamin C but otherwise adequate. However, this factor was not present in sufficient amounts in the orange juice given for its antiscorbutic effects, as neither ascorbic acid nor orange juice had any prophylactic effect on the pathologic changes. They suggested the possibility of some unknown factor, absent from the artificial diet, which may be present in the animal's normal diet containing leafy vegetables and carrots.

Pancreas and yeast both contain lipotropic substances. Best and Ridout[69] attributed the lipotropic effect of pancreas to its content of choline, methionine and inositol. Another unidentified factor, "lipocaic," has also been described (Dragstedt, Van Prohaska and Harms[70]; Van Prohaska, Dragstedt and Harms[71]). Recently, Chaikoff, Entenman and Montgomery[50] suggested that the lipotropic activity of pancreatic extracts is due to the methionine liberated from dietary protein. That yeast can prevent cirrhosis was shown by Webster,[72] who prevented hepatic cirrhosis in rats by administering large amounts of yeast or molasses. However, the nature of its action is unknown, although Himsworth and Glynn[17b] expressed the belief that this is due to its lipotropic property.

Influence of Diet in Modifying Hepatic Injury Due to Hepatotoxic Agents.—Opie and Alford[2] were the first to demonstrate that the degree to which animals were susceptible to chloroform poisoning depended largely on their diet. A high carbohydrate diet afforded considerable protection, while meat was less effective. The protective value of carbohydrates was confirmed in 1919 by Davis and Whipple[3] when they demonstrated definite diminution of resistance to chloroform poisoning in starving animals.

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65. Gavin, G., and McHenry, E. W.: J. Biol. Chem. **141**:619, 1941.

66. Best, C. H.; Lucas, C. C.; Patterson, J. M., and Ridout, J. H.: Biochem. J. **40**:368, 1946.

67. Greene, C. H.: J. A. M. A. **121**:715, 1943

68. Spellberg, M. A., and Keeton, R. W.: Am. J. M. Sc. **200**:688, 1940.

69. Best, C. H., and Ridout, J. H.: Am. J. Physiol. **122**:67, 1938.

70. Dragstedt, L. R.; Van Prohaska, J., and Harms, H. P.: Am. J. Physiol. **117**:175, 1936.

71. Van Prohaska, J.; Dragstedt, L. R., and Harms, H. P.: Am. J. Physiol. **117**:166, 1936.

72. Webster, G.: Tr. A. Am. Physicians **55**:139, 1940.

A high fat diet has an adverse effect on the resistance of the liver. Schifrin[73] reported that hepatic necrosis produced in dogs with arsphenamine was maximum in animals on a high fat diet. Messinger and Hawkins[74] obtained the same results with arsphenamine. Himsworth and Glynn,[17a] while investigating the effect of diet on the livers of rats poisoned with trinitrotoluene, observed that the animals fed a high fat diet fared badly, while those fed diets high in protein or high in carbohydrate enjoyed almost complete protection. Bollman[75] found that when extensive hepatic necrosis was produced in rats by repeated exposure to carbon tetrachloride, the greatest amount of damage was present in fat-fed rats and the least in rats fed carbohydrates. Regenerative changes were most evident in the animals receiving protein diets.

Lipotropic agents have not been found to exert any appreciable protective action against hepatotoxic substances such as chloroform (Miller, Ross and Whipple[76]), phosphorus (Best, MacLean and Ridout[77]) and carbon tetrachloride (Barrett and associates[78]; Post and associates[79]). However, Von Glahn and Flinn[7] reported that yeast added to the diet of rabbits reduces somewhat the tendency of the liver to be damaged by lead arsenate, and Suguira and Rhoads[80] reported protection against para-dimethylaminoazobenzene.

Sometimes a diet with a high fat content seems to exert protective action against necrosis due to deficiency of sulfur-containing amino acids or that due to exogenous poisons (György and Goldblatt[59c]; Himsworth and Glynn[17b]). Turnbull[81] pointed out the tendency of liver cells containing fat to be spared in poisoning with trinitrotoluene. Smith[82] observed that rats fed a high fat diet showed less hepatic injury in experimental poisoning. A similar observation was made by Earle and Victor[83] in hepatic injury produced by a high intake of cystine.

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73. Schifrin, A.: Virchows Arch. f. path. Anat. **287**:175, 1932.

74. Messinger, W. J., and Hawkins, W. B.: Am. J. M. Sc. **199**:216, 1940.

75. Bollman, J. L.: J. A. M. A. **121**:1413, 1943.

76. Miller, L. L.; Ross, J. F., and Whipple, G. H.: Am. J. M. Sc. **200**:739, 1940.

77. Best, C. H.; MacLean, D. L., and Ridout, J. H.: J. Physiol. **83**:275, 1935.

78. Barrett, H. M.; Best, C. H.; MacLean, D. L., and Ridout, J. H.: J. Physiol. **97**:103, 1939.

79. Post, J.; Earle, D. P.; Patek, A. J., Jr., and Victor, J.: Am. J. Path. **18**:661, 1942.

80. Suguira, K., and Rhoads, C. P.: Cancer Research **1**:3, 1941.

81. Turnbull, H. M.: Proc. Roy. Soc. Med. **10**:47, 1917.

82. Smith, M. I.: Pub. Health Rep. **54**:1441, 1939.

83. Earle, D. P., and Victor, J.: J. Exper. Med. **75**:179, 1942.

That protein also plays a part in modifying the damage of the liver has been pointed out recently by Goldschmidt, Vars and Ravdin[84] in rats in which protein diminished the extent of chloroform necrosis. Miller and Whipple[85] noted that the injury produced in the livers of dogs by chloroform anesthesia was directly proportional to protein depletion. Similar observations have been reported in regard to arsphenamine and trinitrotoluene poisoning (Schifrin[73]; Messinger and Hawkins[74]; Himsworth and Glynn[17a]). Smith, Lillie and Stohlman[86] observed that high protein diets protected against para-aminoazobenzene and dimethylaminoazobenzene. Maxon[87] and Smith[82] demonstrated a similar protective action against selenium.

Types of Hepatic Lesions Produced in Animals by Dietary Deficiency.—It is only within the past few years that lesions of the liver resulting from dietary deficiency have attracted attention. The hepatic lesions produced by diet alone or by diet plus poisons, toxins or infections can be grouped as follows:

Necrosis: A massive acute necrosis, either diffuse (simulating acute yellow atrophy of man) or focal, with or without fatty changes, and with or without hemorrhage, has been produced by various workers and is the most striking lesion produced by dietary deficiency (György and Goldblatt[88]; Cameron and Karunartne[89]; Earle and Victor[37]; Himsworth and Glynn[17b]; Glynn and Himsworth[90]). Lesions observed by Weichselbaum[4] in rats fed a low cystine diet were probably of the same nature, although he described them only as hemorrhages. Recent work of Himsworth and Glynn[18] and Glynn, Himsworth and Neuberger[91] indicates that necrosis is not necessarily the sequel to fatty infiltration but is the result of thioaminoacid deficiency.

Fatty Infiltration: This is usually produced by diets low in protein and high in neutral fat (Handler and Bernheim[92]; Glynn[48]) and is probably due to deficiency of choline or of amino acids like methionine

84. Goldschmidt, S.; Vars, H. M., and Ravdin, I. S.: J. Clin. Investigation **18**:277, 1939.

85. Miller, L. L., and Whipple, G. H.: Am. J. M. Sc. **199**:204, 1940.

86. Smith, M. I.; Lillie, R. D., and Stohlman, E. F.: Pub. Health Rep. **58**:304, 1943.

87. Maxon, A. L.: Bulletin 311, South Dakota Agricultural Experiment Station, 1937.

88. György and Goldblatt (footnotes 58 and 59*c*).

89. Cameron, G. R., and Karunartne, W. E.: J. Path. & Bact. **42**:1, 1936.

90. Glynn, L. E., and Himsworth, H. P.: J. Path. & Bact. **56**:297, 1944.

91. Glynn, L. E.; Himsworth, H. P., and Neuberger, A.: Brit. J. Exper. Path. **26**:326, 1945.

92. Handler, P., and Bernheim, F.: J. Biol. Chem. **148**:649, 1943.

which make methyl groups available for the synthesis of choline. It has also been produced in pancreatectomized dogs maintained with insulin (Chaikoff and co-workers[49]) and is probably due to the impairment of digestion and resulting malabsorption. The condition could be prevented by the administration of lecithin.

Diffuse Hepatic Cytosiderosis: This has been produced in rabbits by chronic copper poisoning (Mallory and associates[93]; Mallory[94]; Mallory and Parker[95]) and in cats by ligation of the pancreatic duct (Taylor, Stiven and Reid[96]). The condition is interesting, as cytosiderosis is a constant finding in adult pellagrins in South Africa and is frequently associated with cirrhosis of the liver (Gillman and Gillman[97]; Gillman, Mandelstam and Gillman[98]).

Lobar Hypertrophy With or Without Lobar Absorption, Secondary to Vascular Derangements: This condition has been reported by Gillman and associates.[16]

Generalized Atrophy: This is one of the categories of diseases of the liver produced by the maize meal diet (Gillman and associates[16]).

Cirrhosis: Cirrhosis has been produced experimentally by two distinct procedures: (1) repeated administration of various hepatotoxic agents, such as carbon tetrachloride and manganese (Cameron and Karunartne[89]; Moon[99]), and (2) establishing nutritional deficiencies. In the latter case it may be preceded by severe and prolonged fatty infiltration (Chaikoff and associates[49]; Connor[55]; György and Goldblatt[58]; Spellberg and Keeton[68]; Blumberg and Grady[51]; Handler and Bernheim[92]; Himsworth and Glynn[100]; Gillman and co-workers[16]) or it may follow postnecrotic scarring with or without nodular hyperplasia (György and Goldblatt[88]; Hock and Fink[101]; Himsworth and Glynn[17b]).

An important observation in the pathologic examination of experimental fatty cirrhosis of rats is that of a pigment in the liver (György and

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93. Mallory, F. B.; Parker, F., Jr., and Nye, R. N.: J. M. Research **42**:461, 1921.

94. Mallory, F. B.: Am. J. Path. **1**:117, 1925.

95. Mallory, F. B., and Parker, F., Jr.: Am. J. Path. **7**:365, 1931.

96. Taylor, J.; Stiven, D., and Reid, E. W.: J. Path. & Bact. **34**:793, 1931; **41**:397, 1935.

97. Gillman, J., and Gillman, T.: Arch. Path. **40**:239, 1945.

98. Gillman, J.; Mandelstam, J., and Gillman, T.: S. African J. M. Sc. **10**:109, 1945.

99. Moon, V. H.: Arch. Path. **18**:381, 1934.

100. Himsworth and Glynn (footnotes 17*b* and 18).

101. Hock, A., and Fink, H.: Ztschr. f. physiol. Chem. **279**:187, 1943.

Goldblatt[50e]; Blumberg and Grady[51]; Edwards and White[102]; Popper, György and Goldblatt[103]; Endicott and Lillie[104]; György[105]). This pigment has been named "ceroid" by Lillie, Daft and Sebrell.[8] Victor and Pappenheimer[106] observed a similar acid-fast pigment in various tissues, including the livers, of certain patients with nutritional disorders and hepatic disease. They pointed out the possibility that the pigment was related to vitamin E deficiency. Popper, György and Goldblatt[103] suggested that ceroid probably arises from fat globules in cells deficient in vitamin E and that its formation is favored by absorption factors and inhibited by lipotropic factors (Pappenheimer and Victor[107]). However, they[103] were not able to demonstrate this pigment in normal or in cirrhotic human livers, nor has it been observed in dietary deficiency cirrhosis in dogs (Chaikoff and co-workers[108]). In human alcoholic cirrhosis, which is not recognized as based on a deficient diet, Mallory[109] described eosinophilic hyaline bodies in the liver cells undergoing degeneration as a characteristic finding. These have not been observed in the experimental cirrhosis of rats.

From the foregoing review it is apparent that hepatic injury can be produced in animals by various methods and that this injury when severe or prolonged may terminate in cirrhosis. It is hardly an exaggeration to say that practically every dietary factor save carbohydrate has, at one time or another, been credited with playing a role in the production of hepatic injury, and the uncertainty in this field is so great that Bollman[110] in a recent review wrote that dietary injury of the liver could apparently be brought about in many different ways.

Summary of Experimental Work.—Two major types of hepatic injury have been produced in animals by feeding them deficient diets. The one is fatty infiltration of the liver, which, if pronounced and sustained, may develop into cirrhosis of Laennec's type. The other acute necrosis (comparable to acute yellow atrophy of man), which, if not fatal, results in scarring and nodular hyperplasia. The two lesions may be present at the same time, and this sometimes makes it difficult to place them in

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102. Edwards, J. E., and White, J.: J. Nat. Cancer Inst. **2**:157, 1941.

103. Popper, H.; György, P., and Goldblatt, H.: Arch. Path. **37**:161, 1944.

104. Endicott, K. M., and Lillie, R. D.: Am. J. Path. **20**:149, 1944.

105. György, P.: Am. J. Clin. Path. **14**:67, 1944.

106. Victor, J., and Pappenheimer, A. M.: J. Exper. Med. **82**:375, 1945.

107. Pappenheimer, A. M., and Victor, J.: Am. J. Path. **22**:402, 1946.

108. Chaikoff, I. L.; Eichron, K. B.; Connor, C. L., and Entenman, C.: Am. J. Path. **19**:9, 1943.

109. Mallory, F. B.: Bull. Johns Hopkins Hosp. **22**:69, 1911.

110. Bollman, J. L., in Luck, J. M.: Annual Review of Physiology, Stanford University, Calif., Annual Reviews, Inc., 1943, vol. 5, p. 321.

separate categories. That fatty infiltration may eventually lead to cirrhosis was first observed in depancreatized dogs kept alive with diet and insulin. It was then observed that the cirrhosis could be prevented by feeding the animals lecithin, choline and pancreas. The action of the latter may be due to the lecithin which it contains or to lipocaic. The common property of all these substances is their lipotropic activity. On the basis of this theme, an effort was made to produce fatty infiltration by feeding animals a high fat diet. After several months, fatty infiltration and cirrhosis of the portal type developed in these animals. Fatty infiltration acted by causing anoxemia and alterations of cell metabolism, leading to death of liver cells. It was further observed that in the early stages the process was reversible and that if the fat content of the liver could be reduced to normal by the administration of lipotropic substances, e.g., lecithin and choline, the hepatic damage could be prevented from developing into cirrhosis.

Later it was found that while cystine was ineffective, casein or methionine added to the diet prevented fatty infiltration in spite of a high fat content. The action of casein is probably due to one of its component amino acids, methionine. Choline and methionine have in common the methyl group (CH_3) which cystine does not contain. Witts[111] has well illustrated the action of lipotropic agents which centers around the supply of labile methyl groups:

Pancreas → lecithin → choline → labile
Casein → methionine → labile methyl group

When there is deficiency of labile methyl groups, the body is unable to manufacture lecithin, which is required for the transfer of the hepatic fat. The fat accumulates and damages the liver cell by causing anoxemia and alterations of cell metabolism. The ultimate death of cells is followed by avascular fibrosis.

Himsworth and Glynn[17a] showed that protein deficiency, besides causing fatty infiltration, may cause acute damage in the form of necrosis developing within a few weeks. They found that different proteins vary in their ability to prevent necrosis; e.g., casein is effective in small amounts while yeast is ineffective even in large quantities. The main difference between the two proteins is that yeast is poor in sulfur-containing amino acids. The development of the acute necrotic lesions may be prevented if the rats are fed the two sulfur-containing amino acids, methionine and cystine. These observations suggested that the sulfydryl (SH) group is as essential as the methyl group (CH_3) for the prevention of dietary cirrhosis (Glynn, Himsworth and Neuberger[91]). Methionine, which contains both methyl and sulfhydryl groups, is the key substance in the metabolism of the liver and is one of the most essential amino acids. On

111. Witts, L. J.: Brit. M. J. 1:1, 1947.

the other hand, an excess of cystine may be harmful, and Earle and Victor[83] have produced lesions of the necrotic type by giving 300 mg. of cystine per day.

The relation of the vitamin B complex to cirrhosis and necrosis has yet to be clearly determined. Some of the B vitamins, particularly biotin, favor the deposition of fat in the rat's liver. On the present evidence it seems justifiable to conclude that the massive dose of yeast or of any other source of the vitamin B complex is not likely to produce ill effects on the liver in hepatic diseases of man (Witts[111]), although the beneficial effects of such a therapeutic regimen are not clearly established.

The effect of diets high in carbohydrate is not clear. It has long been known that in hepatic damage depletion of glycogen takes place and that animals fed a high carbohydrate diet are more protected against drugs which produce fatty infiltration of the liver than are those given a diet high in fat (Rosenfeld[112]). A high carbohydrate diet affords some protection against chloroform poisoning and is of value in the prevention of the hepatic damage following experimental ligation of the common bile duct, the making of Eck's fistula, mushroom poisoning and partial hepatectomy (Soskin and Hyman[113]). The action of carbohydrates is that of fuel for the hepatic cells in their work of transport, protein synthesis and detoxication. The carbohydrates probably act through their sparing of proteins, but they cannot protect the liver unless the requirements of protein metabolism and total calories are satisfied (Witts[111]). If these requirements are not met, an unbalanced carbohydrate diet may actually favor the development of necrosis (Craven[114]).

CIRRHOSIS OF THE LIVER IN HUMAN SUBJECTS

Incidence.—Ratnoff and Patek[115] have given a comprehensive analysis of the world incidence of Laennec's cirrhosis as encountered in autopsy material. They place the highest incidence of cirrhosis in China (12.0 per cent), Zurich, Switzerland (10 per cent), Vizagapatam in South India (9.3 per cent), London, England (7.7 per cent) and Philadelphia (6.1 per cent). In East Africa, Vint[116] reported cirrhosis as observed in 6.7 per cent of the autopsies performed on natives. In the majority of the other countries the incidence varies from 1 to 4.5 per cent.[115]

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112. Rosenfeld, G.: Ergebn. d. Physiol. **2**:50, 1903.

113. Soskin, S., and Hyman, M.: Arch. Int. Med. **64**:1265, 1939.

114. Craven, E. B., Jr.: Bull. Johns Hopkins Hosp. **48**:131, 1931.

115. Ratnoff, O. D., and Patek, A. J., Jr.: Medicine **21**:207, 1942.

116. Vint, F. W.: East African M. J. **7**:349, 1931.

Cirrhosis is more common in Indians than is usually appreciated, and the high incidence has been constantly recorded by Rogers,[117] Hance,[118] Hughes[119] and Tirmurti and Rao.[120] The figures quoted by Ratnoff and Patek[115] for various parts of India are: Calcutta, 6.9 per cent; Madras, 4.4 per cent; Vizagapatam, 9.3 per cent.

Cirrhosis of the liver is thus encountered in all the countries of the world but is most common in China, South Africa, East Africa, India, Ceylon and Java.

Role of Alcohol and Diet in the Causation of Human Cirrhosis.—It can now be assumed that evidence has accumulated showing that cirrhosis of the liver may have its genesis in some form of nutritional deficiency.

Alcoholism: Alcohol as a direct cause of cirrhosis has been pretty well dismissed (Jolliffe and Jellineke[121]), although when an excessive intake of alcohol is associated with an insufficient diet, fatty infiltration is frequently noted (Connor[55]). In a review of experimental cirrhosis in laboratory animals, Moon[99] cited several workers who failed to produce cirrhosis by feeding animals on alcohol. Friedenwald[122] failed to produce cirrhosis in rabbits by administering 5 to 8 cc. of absolute alcohol diluted in 20 to 30 cc. of water daily over a period of four years.

Cirrhosis of the liver is common in India, Java and Ceylon, where alcoholism is rare. Tirmurti and Rao,[120] Menon and Annamalai,[123] Hughes[119] and Manson-Bahr[124] concluded, after a detailed survey, that alcohol could not be the cause of cirrhosis in Indians. Thus, in Vizagapatam, South India, portal cirrhosis was seen in 5.2 per cent of all the autopsies, but only 3 per cent of the patients with portal cirrhosis had been addicted to the consumption of alcoholic beverages (Sutherland[125]). This opinion has been confirmed by Yenikomshian[126] in Lebanon and Syria, and by Stacey[127] in Iraq.

117. Rogers, L.: (*a*) Indian M. Gaz. **46**:47, 1911; (*b*) Lancet **2**:355, 1912; (*c*) Brit. M. J. **1**:345, 1922.

118. Hance, J. B.: Guy's Hosp. Rep. **78**:379, 1928.

119. Hughes, T. A.: Indian J. M. Research **21**:353, 1933.

120. Tirmurti, T. S., and Rao, M. V. R.: Indian M. Gaz. **69**:74, 1934.

121. Jolliffe, N., and Jellineke, M.: Quart. J. Stud. on Alcohol **2**:544, 1941.

122. Friedenwald, J.: J. A. M. A. **45**:780, 1905.

123. Menon, T. B., and Annamalai, D. R.: Indian J. M. Research **22**:827, 1935.

124. Manson-Bahr, P. H. L.: Manson's Tropical Diseases: A Manual of the Diseases of Warm Climates, ed. 12, London, William Wood & Company, 1945.

125. Sutherland, D. W.: Indian M. Gaz. **40**:121, 1905.

126. Yenikomshian, H. A.: J. A. M. A. **103**:660, 1934.

127. Stacey, R. S.: Tr. Roy. Soc. Trop. Med. & Hyg. **37**:387, 1944.

Dietary Factors: Workers in the East (Yang[128]; Rao[129]; Yenikomshian[126]; Tyagaraja[130]) had suggested that dietary factors were concerned in the causation of hepatic cirrhosis, but diet was never considered of prime importance. Malaria, dysentery and parasitic infections were regarded as directly responsible for the production of cirrhotic livers, with diet playing only a contributory role.

It has long been felt that in Indians cirrhosis of the liver is the result, at least in part, of malnutrition, which is widely prevalent both in acute and chronic forms, owing to poverty and religious restrictions (Hughes[131]; Hughes and Shrivastava[132]). Witts[111] mentioned the differences in the incidence of, and the mortality from, infective hepatitis in the India Command during 1944, from the figures collected by Lt. Col. Stuart McDonald. The incidence of jaundice was five times as high in the British troops as in the Indian troops, but the mortality was just the reverse. The factor favoring the low incidence of jaundice could be a nutritional difference or a previous exposure, but it is almost certain that the high mortality of Indians with this disease was due to malnutrition.

At the Central Command Conference [133] on anemias in Agra, India, it was definitely brought out by various pathologists that anemia and malnutrition were prevalent in Indian troops. The conditions prevailing among the Indian civilians are similar. The most common type of anemia encountered in Indians with cirrhosis is the normochromic or hypochromic macrocytic type, due primarily to malnutrition and curable by improved nutrition with or without any change in the pathologic condition of the liver.

Nutritional deficiency is also claimed to play a part in the production of cirrhosis in Indian children. Rao[134] reported that cirrhosis is found mostly in the children of vegetarian parents, especially in those infants who are weaned early and who are fed either cow's milk or the family vegetarian diet. It could be prevented by feeding the infants modern patented milks. In a case reported by him, a mother of twins, a boy and a girl, faced with the choice of breast feeding one of them only, weaned the girl and fed her cow's milk. Cirrhosis developed in the girl, while the boy remained healthy.

128. Yang, C. S.: Nat. M. J. China **14**:195, 1928.

129. Rao, M. V. R.: Indian J. M. Research **21**:389, 1933.

130. Tyagaraja, S.: Ceylon J. Sc., Sect. D., M. Sc. (pt. 3.) **4**:119, 1937.

131. Hughes, T. A.: Indian J. M. Research **14**:157, 1926. Hughes[119]

132. Hughes, T. A., and Shrivastava, D. L.: Indian J. M. Research **15**:427, 1927.

133. Proceedings of the Central Command Conference on Anemia, Agra, India, Nov. 20-21, 1944.

134. Rao, P. K.: Proc. Indian Acad. Sc., Sect. B **14**:310, 1941.

Gillman[14] reported cirrhosis of the liver to be widely prevalent in the poor natives of the Rand in South Africa, who eat bulky vegetarian diets. They commonly present a condition known as kwashiorkor, characterized clinically by edema, pellagrous lesions of the skin, steatorrhea and microcytic anemia (Trowell and Muwazi[135]). Biopsy or postmortem examination of the liver reveals extreme fatty infiltration (Smith[136]), and there is ample evidence that this is followed by cirrhosis.

During the war the colored troops and the native races of the Middle East and the East showed a high mortality from infective hepatitis. Witts[137] reported an outbreak in the British Colonial troops with a mortality of 40 per cent. In 1943 a high mortality was recorded in a small epidemic among poorly nourished African laborers in Uganda. Of the 14 patients admitted to the hospital, all died (Witts[111]).

In Jamaica, infantile cirrhosis of the liver is common, particularly in the children of unmarried mothers who support themselves and their babies by working. Breast feeding is not too common; the infants are fed mostly on a diet consisting of carbohydrates, arrowroot, cereal, gruel or even sugar and water. It has also been observed that cirrhosis is less frequent in the fishing villages, where the protein content of the diet may be higher (McFarlane and Branday[138]; Platt[139]; Witts[111]).

Foods grown on soil with a high selenium content have been reported to cause severe injury of the liver, which may develop into cirrhosis (Moxon and Rhian[140]). These authors pointed out that persons subsisting on high carbohydrate, low protein diets are more liable to get selenium cirrhosis than those on high protein diets, and concluded that it is the badly balanced diet which prepares the ground for the toxic action of selenium.

Role of Infection in the Causation of Human Cirrhosis.—Since parasitic infections are so widespread in the countries where cirrhosis is most prevalent, attention has been constantly focused on elucidating the part played by parasitic and other infections in the production of cirrhosis.

The rather frequent background of malaria in Indians has been responsible for constant speculation regarding the possibility that malaria is related to cirrhosis. Sitsen,[141] Hance[118] and Hughes[119] presented ev-

135. Trowell, H. C., and Muwazi, E. M. K.: Arch. Dis. Childhood **20**:110, 1945.

136. Smith, E. C.: Tr. Roy. Soc. Trop. Med. & Hyg. **36**:287, 1943.

137. Witts, L. J.: Brit. M. J. **1**:739, 1944.

138. McFarlane, A. L., and Branday, W. J.: Brit. M. J. **1**:838, 1945.

139. Platt, B. S.: Nutrition in British West Indies, Great Britain Colonial no. 195, London, His Majesty's Stationery Office, Oct. 9, 1945.

140. Moxon, A. L., and Rhian, M.: Physiol. Rev. **23**:305, 1943.

141. Sitsen, A. E.: Geneesk. tidjschr. v. Nederl.-Indië **62**:5, 1921; abstracted, Trop. Dis. Bull. **20**:22, 1922.

idence in support of the view that malarial necrosis of the liver is an important cause of cirrhosis. Rogers[142] reported that he did not come across malarial cirrhosis in a single one of a series of 5,000 autopsies in Calcutta, where malaria is endemic. Tirmurti and Rao[143] reviewed the role of malaria in cirrhosis and studied the livers of malarial subjects. They concluded that there was no evidence from pathologic material that malaria produces cirrhosis. Osler[144] did not come across a single person with malarial cirrhosis in the wards or the autopsy room at the Johns Hopkins Hospital over a period of fifteen years. Stacey[127] found no evidence of malaria either clinically or at postmortem examination in cases of cirrhosis in Iraq.

Amebiasis leading to amebic hepatitis has been constantly blamed as causing cirrhosis of the liver in the tropics. Rogers[117a] propounded the view that the portal cirrhosis of Indians resulted commonly from amebiasis. His explanation was that focal and diffuse necrosis of liver tissue caused by amebic infection results in fibrosis. Rogers,[145] Menon and Annamalai,[123] Rao[129] and Wang[146] reported the incidence of dysentery in patients with cirrhosis in India and China as from 25 to 40 per cent, compared with 7 to 17 per cent in the general population. Tirmurti and Rao[147] found no evidence of active or old dysentery in postmortem examinations of 18 persons with cirrhosis of the liver. The same authors,[120] discussing the role of amebiasis, argued that the fibrosis occurring around the amebic abscesses was localized and could not be regarded as the cause of diffuse portal fibrosis. In examining biopsy specimens of livers from patients with chronic amebic hepatitis at Agra, I never came across a definite appearance of portal cirrhosis, although postnecrotic scarring was not infrequent. However, protracted amebiasis with intestinal complications would undoubtedly impair nutrition—a result which would be grave in patients who are already ill fed. It could be a precipitating factor in a liver which has already been damaged by malnutrition.

Schistosomiasis has been claimed as a cause of cirrhosis in Egypt (Day[148]), where it is endemic. However, in India and China (Yang[128]) this type of infection is absent and cannot be considered the cause of cirrhosis. In the series reported by Stacey[127] from Iraq, there is no evidence that schistosomal infection is the cause of cirrhosis there.

142. Rogers, L.: Practitioner **131**:117, 1933.

143. Tirmurti, T. S., and Rao, M. V. R.: Indian J. M. Research **24**:149, 1936.

144. Osler, W.: The Principles and Practice of Medicine, ed. 7, Philadelphia, D. Appleton and Company, 1909.

145. Rogers (footnotes 117*c* and 142).

146. Wang, C. F.: Chinese M. J. **50**:891, 1936.

147. Tirmurti, T. S., and Rao, M. V. R.: J. Indian M. A. **1**:423, 1932.

148. Day, H. B.: Tr. Roy. Soc. Trop. Med. & Hyg. **18**:121, 1924.

Bacterial and virus infections have been suspected of playing a primary or a precipitating casual role in the production of cirrhosis, especially in children, in whom the illness is usually ushered in by fever. Cirrhosis has been reported occurring in several children of the same family. In all these it usually has been preceded by some acute infection. Ely[149] reported cirrhosis in 4 year old twins whose symptoms began with jaundice which developed during an attack of measles and ended five months later in death. Moon[150] reported cirrhosis occurring in 3 members of one family, following scarlet fever. Hemolytic streptococci were cultivated from the liver of 1 at postmortem examination. The streptococci were inoculated into 12 young rabbits by various routes. The animals lived three to fifteen days, and the hemolytic streptococci were recultured from the livers of 11; the livers showed marked degeneration and necrosis. Moon[150] also cultivated streptococci from the liver in 6 cases of cirrhosis and demonstrated cocci in sections in 9 others in which the cirrhotic process was active. McMahon and Mallory[151] reported that beta hemolytic streptococci were demonstrated in 4 of 5 cases of streptococcic hepatitis.

Livers infected with Escherichia coli (McMahon[152]), Staphylococcus aureus (Phillipps[153]) or Treponema pallidum (McMichael[154]) have also been reported to show a similar picture. Rao[134] reported that Bacillus coli had been cultured from liver in 1 of his cases. Harrell and McBryde[155] in a recent review concluded that dietary deficiency combined with repeated infection is an important etiologic factor of the cirrhosis developing in children. In most of these the condition was probably hepatic necrosis followed by postnecrotic scarring and nodular hyperplasia.

Clinical Application of Experimental Observations.—The medical literature of recent years contains a large number of reports of cases in which disease of the human liver was treated with specific diets. These cases can be grouped into two major categories: cases of chronic hepatic cirrhosis and cases of acute infectious and toxic hepatitis.

Patek[156] observed that in cases of cirrhosis associated with alcoholism high vitamin therapy led to improvement of hepatic function. Lewis,

149. Ely, T.: Boston M. & S. J. **170**:542, 1914.
150. Moon, V. H.: Am. J. M. Sc. **177**:681, 1929.
151. McMahon, H. E., and Mallory, F. B.: Am. J. Path. **7**:299, 1931.
152. McMahon, H. E.: Am. J. Path. **7**:77, 1931.
153. Phillipps, F. A.: Lancet **1**:1050, 1937.
154. McMichael, J.: J. Path. & Bact. **39**:481, 1934.
155. Harrell, G. T., and McBryde, A.: Am. J. Dis. Child. **59**:1301, 1940.
156. Patek, A. J., Jr.: Proc. Soc. Exper. Biol. & Med. **37**:329, 1937.

Taylor and Davidson[157] obtained satisfactory results in 19 patients with portal cirrhosis by the use of a "liver protein digest ("ledinac")"—a granular powder prepared by vacuum drying of a partial enzymatic (papain) hydrolysate of the beef liver pulp remaining after hot aqueous extraction of the antipernicious anemia factor. The preparation contains 60 per cent protein in addition to large quantities of the known components of the vitamin B complex.

Working on the experimental evidence that the absence of lipotropic amino acids produces fatty infiltration of the liver, Fagin and Zinn[158] reported encouraging results in patients treated parenterally with amino acids. Franklin and co-workers[159] studied the histologic changes in the livers by repeated biopsies before and after institution of "lipotropic therapy" (cystine plus choline, methionine and high protein, high vitamin B complex diet). They found disappearance of fat and regeneration of parenchymal cells in all human fatty cirrhotic and noncirrhotic livers. This change was associated with clinical and functional improvement. However, they could not find any evidence of arrest of the cirrhotic process after the removal of fat. This disappearing of fat without concomitant decrease of fibrous tissue after lipotropic therapy has also been reported by Gillman and Gillman[160] and Beams and Endicott.[161] These observations are in accord with clinical findings of Weir[162] and Beams.[163]

Morrison[164] adopted the therapeutic regimen of a high protein, low fat, moderate carbohydrate diet, frequent feedings of skimmed milk and methionine, choline chloride, and a special liver extract containing the vitamin B complex, with favorable results. In recent years various other workers[165] have reported that patients with cirrhosis of the liver improve

157. Lewis, J. H.; Taylor, F. H. L., and Davidson, C. S.: New England J. Med. **236**:351, 1947.

158. Fagin, I. D., and Zinn, F. T.: J. Lab. & Clin. Med. **27**:1400, 1942.

159. Franklin, M.; Salk, M. R.; Steigmann, F., and Popper, H.: Am. J. Clin. Path. **18**:273, 1948.

160. Gillman, T., and Gillman, J.: Arch. Int. Med. **76**:63, 1945.

161. Beams, A. J., and Endicott, E. T., cited by Franklin, M.; Salk, M. R.; Steigmann, F., and Popper, H.: Am. J. Clin. Path. **18**:273, 1948.

162. Weir, J.: J. A. M. A. **134**:579, 1947.

163. Beams, A. J.: J. A. M. A. **130**:190, 1946.

164. Morrison, L. M.: J.A.M.A. **134**:673, 1947.

165. (*a*) Patek, A. J., Jr., and Post, J.: J. Clin. Investigation **20**:481, 1941. (*b*) Fleming, R. G., and Snell, A. M.: Am. J. Digest. Dis. **9**:115, 1942. (*c*) Broun, G. O., and Muether, R. O.: J. A. M. A. **118**:1403, 1942. (*d*) MacKenzie, C. G., and Seligson, D.: Federation Proc. **1**:187, 1942. (*e*) Daft, F. S.; Sebrell, W. H., and Lillie, R. D.: Proc. Soc. Exper. Biol. & Med. **50**:1, 1942. (*f*) Hoagland, C. L.: New York State J. Med. **143**:1041, 1943. (*g*) Russakoff, A. H., and Blumberg, H.: Ann. Int. Med. **21**:848, 1944. (*h*) Rimmerman, A. B.;

or recover after receiving diets rich in protein or in one or the other of its component amino acids, especially choline and cystine, or methionine.

Lately the trend has been to use various combinations of diets high in protein with a high carbohydrate and a moderate to low fat content, plus fortified casein hydrolysate, dried brewers' yeast orally, liver extract parenterally, and specific vitamins and amino acids when these are indicated (Patek[156]; Patek and Post[165a]; Fleming and Snell[165b]; Butt and Snell[166]; Brown and Muether[165c]; Hoagland[165f]; Jollife and Alpert[167]; Morrison[165k]; Lewis and co-workers[157]; Wade[168]). The most promising results were observed in cases of enlarged liver, in which the enlargement was probably due to fatty infiltration, which disappeared after the use of lipotropic agents. Once fibrosis develops, it is hardly possible to effect any improvement, though recently Sellers[169] has shown in rats an apparent decrease in fibrosis after lipotropic activity.

Patients have been treated for infectious hepatitis with methionine, with fairly good results (Beattie and Marshall[170]; Eddy [171]). On the other hand, Wilson and associates[172] and Higgins and Associates[173] have reported that the results obtained with methionine in epidemic hepatitis were disappointing. Following the suggestion of Glynn, Himsworth and Neuberger[91] that cystine was more potent in preventing hepatic necrosis, Wilson, Pollock and Harris[174] treated patients for infectious hepatitis with cystine and found a significant decrease in the relapse rate.

Results with the use of methionine in toxipathic hepatitis have been reported by several investigators—in carbon tetrachloride poisoning by Eddy[175] and Beattie and co-workers,[176] in hepatitis due to trinitrotoluene

Schwartz, S. O.; Popper, H., and Steigman, F.: Am. J. Digest. Dis. **11**:401, 1944. (*i*) Barker, W. H.: M. Clin. North America **29**:273, 1945. (*j*) Ralli, E. P.; Robson, J. S.; Clark, D. H., and Hoagland, C. L.: J. Clin. Investigation **24**:316, 1945. (*k*) Morrison, L. M.: Ann. Int. Med. **24**:465, 1946. (*l*) Patek, A. J., Jr.: New York State J. Med. **46**:2519, 1946. (*m*) J. Mt. Sinai Hosp. **14**:1, 1947.

166. Butt, H. R., and Snell, A. M.: Proc. Staff Meet., Mayo Clin. **17**:250, 1942.

167. Jolliffe, N., and Alpert, E.: M. Clin. North America **29**:655, 1945.

168. Wade, L. J.: M. Clin. North America **29**:479, 1945.

169. Sellers, E. A.: Federation Proc. **6**:290, 1947.

170. Beattie, J., and Marshall, J.: (*a*) Nature, London **153**:525, 1944; (*b*) **154**:547, 1944.

171. Eddy, J. H.: Am. J. M. Sc. **210**:374, 1945.

172. Wilson, C.; Pollock, M. R., and Harris, A. D.: Brit. M. J. **1**:399, 1945.

173. Higgins, G.; O'Brien, J. R. P.; Peters, R. A.; Stewart, A., and Witts, L. J.: Brit. M. J. **1**:401, 1945.

174. Wilson, C.; Pollock, M. R., and Harris, A. D.: Lancet **1**:881, 1946.

175. Eddy, J. H.: J. A. M. A. **128**:994, 1945.

176. Beattie, J.; Herbert, P. H.; Wechtel, C., and Steele, C. W.: Brit. M. J. **1**:209, 1944.

by Eddy[171] and in jaundice complicating arsphenamine therapy by Eddy[171] and in jaundice complicating arsphenamine therapy by Beattie and Marshall[170a] and by Peters and associates.[177]

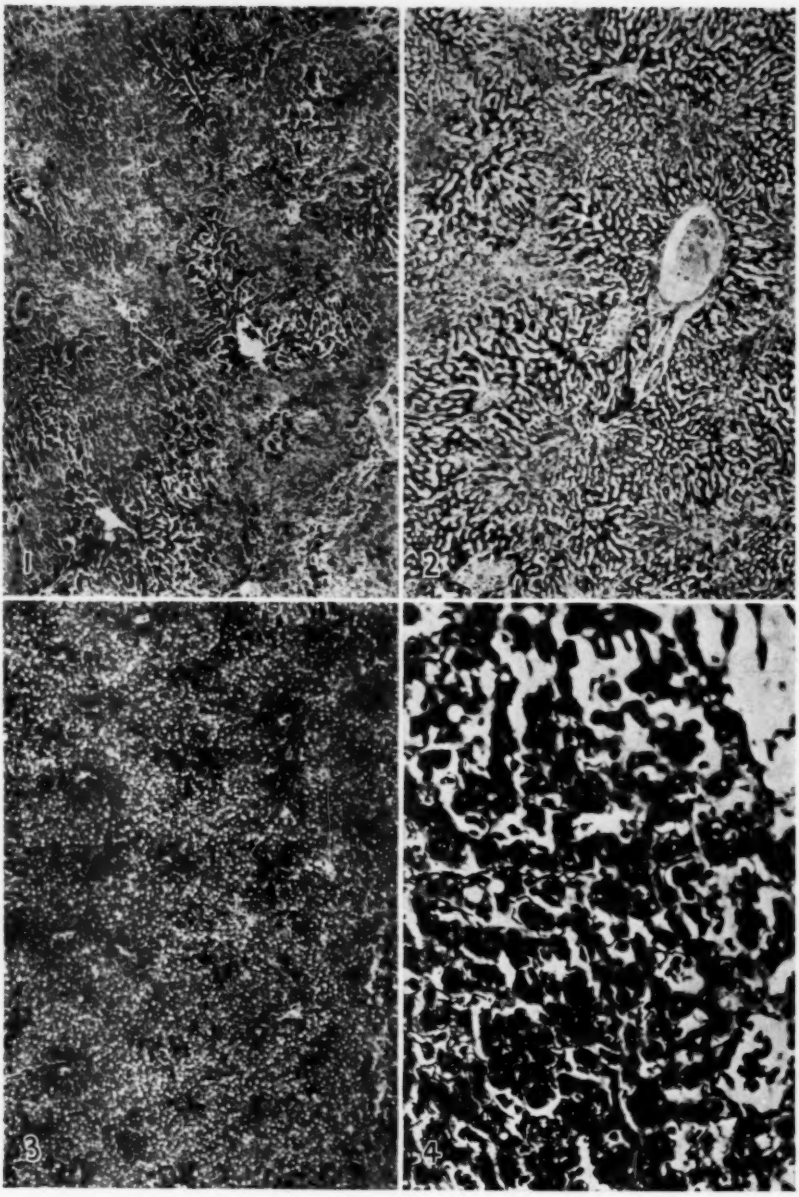
Although animal experiments have clarified the probable etiologic roles of various factors of cirrhosis of the liver, there are many obscure and unexplained things in the genesis of cirrhosis and other hepatic lesions which must be considered before applying the results of animal experiments to man. The most significant contribution of the dietary experiments on animals has been the appreciation of the effect of diet in modifying the degree to which the liver reacts to various hepatotoxic agents. That injury of the liver can be produced by dietetic means is an accepted fact, but the factors responsible are still a source of debate. Every dietetic factor, at one time or another, has been implicated. One must remember, when applying the results to clinical usage, that most of the experiments in animals at present are conducted with synthetic diets which are highly purified and consequently decidedly different from the natural diets of the natives among whom cirrhosis is common. From this point of view, Gillman[14] made the first major contribution toward the understanding of the relation between diet and human cirrhosis. He investigated the effect on rats of prolonged feeding of the staple diet (mealie pap) of the South African natives and found that nodular cirrhosis developed in the rats.

Many are inclined to attribute the high incidence of cirrhosis in Indians to a racial or a genetic factor. They refuse to consider environment as enhancing the sensitivity of a race to the factors productive of hepatic injury. This opinion is based on the fact that cirrhosis of the liver is found particularly in the pigmented people, e.g., Javanese, Indians, Chinese, Japanese and South Africans. It must, however, be remembered that another feature common to these, besides the pigment, is the poverty which compels them to subsist on an unbalanced diet composed of limited amounts of the cheapest foods. However, it is also conceivable that in these countries infections like malaria, amebiasis and schistosomiasis may be responsible for giving the final blow to an already impoverished liver.

OBJECTIVES OF THE PRESENT RESEARCH

This paper represents a preliminary report on one aspect of a comprehensive program initiated at the New England Deaconess Hospital, Boston, in which the effects of various diets on laboratory animals are being investigated with a view to determining the exact role played by diet in the production of cirrhosis of the liver in Indian adults and in-

177. Peters, R. A.; Thompson, R. H. S.; King, A. J.; Williams, D. I., and Nicol, C. S.: Nature, London **153**:773, 1944.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fants. This work will be continued in the pathology laboratory of the Agra Medical College. In subsequent papers the cirrhosis of the liver found in Indians will be discussed and compared with the experimental cirrhosis of rats from the points of view of pathology and histology.

METHODS AND MATERIALS

Animals.—Two sets of albino "Wistar" rats were used in the present experiment. One set consisted of newly weaned rats weighing approximately 50 Gm. These were taken to study the effect of deficient diets on infant livers, as infantile cirrhosis is common in India. The second set of rats weighed approximately 100 to 150 Gm. at the beginning of the experiment. All the animals in one experimental group were of the same sex and approximate weight. The older ones were kept in single cages, while the infant rats were grouped according to the types of diet they were receiving. Once a week the animals were weighed, the older ones individually and the younger ones in groups. In the case of the latter the average weight of the animals was determined.

Feeding.—The animals were fed at the same time each day. The older rats were given 8 Gm. of food each, and the younger ones 5 Gm., irrespective of the nature of the diet. Their consumption of water was not restricted. Before each feeding the residue of the previous meal was collected and weighed. As the diets were moistened with water before administration, allowance was made for water when the amount of foot eaten was calculated.

Diets.—The diets, which were similar to the Himsworth-Glynn[17b] carbohydrate diet, were divided into two groups, one containing 7 per cent yeast and the other 4 per cent casein. The high carbohydrate, low protein, low fat diets are the nearest to the diets of vegetarian Hindus, among whom most cases of cirrhosis occur.

The ingredients of the diets included:

Carbohydrates: Crushed maize starch was used.

Fat: Lard was used as the source of fat.

Yeast: Brewer's yeast no. 1 of the Vitamin Food Company was used. The powder contained protein 49.7 per cent, fat 2.8 per cent, carbohydrate 36.7 per cent, minerals 7.6 per cent and moisture 3.2 per cent.

Casein: Glaxo Laboratories grade A casein was used.

Salt mixture: All the diets contained a salt mixture (3 per cent) made up of potassium iodide 1 per cent, calcium phosphate 1 per cent and sodium chloride 98 per cent.

Fig. 1.—Section of rat's liver showing areas of necrosis. Evidence of post-necrotic scarring is not seen microscopically, but quantitative determination revealed an increase of fibrous tissue. Hematoxylin and eosin; × 50.

Fig. 2.—Section of rat's liver showing distended sinusoids and flattened liver cells. Hematoxylin and eosin; × 50.

Fig. 3.—Section of rat's liver showing extensive fatty infiltration. Hematoxylin and eosin; × 50.

Fig. 4.—Section of rat's liver stained for reticulin fibers. Condensation and thickening of reticulum can be seen. The fibrous tissue was quantitatively determined as 0.9 per cent of the net weight of the liver. Foot's silver stain; × 325.

Vitamins: All the diets contained cod liver oil (1 per cent). A daily supplement of water-soluble vitamins was given to each animal as follows—thiamine hydrochloride 20 mg., riboflavin 20 mg., pyridoxine 10 mg., calcium pantothenate 100 mg. and nicotinamide 50 mg. These were added to the food just before it was given to the animals.

TABLE 1.—*Composition of the Two Types of Diet*

Carbohydrate-Yeast-Protein Diet	Per Cent	Carbohydrate-Casein-Protein Diet	Per Cent
Salt mixture	3	Salt mixture	3
Cod liver oil	1	Cod liver oil	1
Lard	5	Lard	5
Maize starch	84	Maize starch	87
Yeast protein	7	Casein	4
Vitamin supplement		Vitamin supplement	

Histologic Examination.—Liver samples were fixed in formaldehyde-saline and Zenker-acetic acid solutions. Pieces of liver were taken from the middle of the left and the middle of the right lobe, and from any other part which aroused a suspicion of pathologic change. Sections were stained with hematoxylin and eosin and with phloxine-methylene blue. Besides these, sections from each paraffin block were stained with Masson's trichrome stain, Van Gieson's stain for fibrous tissue and Foot's silver stain for reticulum fibers. Frozen sections were stained for fat with scarlet red.

Quantitative Estimation of Fibrous Tissue.—The quantitative determination of the fibrous tissue in normal and pathologic livers of rats was done by the method of Lowry and associates.[178] This method has been of great help in detecting an increase of fibrous tissue in livers in which histologic examinations did not reveal definite microscopic changes. Working with human livers, Warren and Wahi[179] found that the chemical determination is a more accurate method of detecting the presence and the extent of fibrosis in liver than the histologic examination.

Estimation of Hepatic Fat.—This was carried out in accordance with the method described by Leathers and Raper.[180]

RESULTS

The behavior of the animals on carbohydrate diets provided some interesting observations. In the beginning, all the animals seemed to thrive, taking interest in the surroundings, eating well and gaining weight after an initial fall. Then seemingly normal animals would suddenly fall ill and succumb, or would rally only to relapse again after a few weeks.

178. Lowry, O. H.; Gilligan, D. R., and Katersky, E. M.: J. Biol. Chem. **139**:795, 1941.

179. Warren, S., and Wahi, P. N.: Arch. Path. **44**:563, 1947.

180. Leathers, J. B., and Raper, H. S.: The "Fats," London, Longmans, Green & Company, 1925.

After one or a number of such attacks, all the animals died. The first indication of their illness was a loss of appetite. The animal would be huddled in a corner of the cage, and the food remained untouched. If the illness was severe but not fatal, the rat remained ill, ate little and continued to lose weight. If it survived this period, or if the illness was not acute, it became more lively and bgan eating, and its weight became stationary. However, after a second or a third severe attack, it would succumb.

Infant rats succumbed earlier, and all were dead in forty to seventy-seven days. Adult rats showed better resistance, the first one dying after one hundred days. After this they died at various intervals corresponding to the various episodes of illness. Rats on microscopic examination showing changes leading ultimately to fibrosis had an almost uneventful clinical course. They retained their activity and appetite to the end. The only indication of something wrong was an erratic weight curve showing rapid falls and equally rapid recoveries. If not killed, the animal usually died of some indefinite illness. Fatty infiltration of the liver was seen in the animal which died on the hundredth day, but early cirrhotic changes were present only in animals living longer.

PATHOLOGIC CHANGES

Livers of Rats Weighing 50 Gm.—The infant rats showed changes which were different in many respects from those observed in the adults Neither showed gross or microscopic changes characteristic of portal cirrhosis (diffuse hepatic fibrosis) as described by Himsworth and Glynn.[17b] On microscopic examination, the liver was swollen and reddish purple, and petechiae were present. One rat which survived a few weeks after a severe attack of illness had a reddish yellow liver which was fairly smooth. It also had ascites. In none of the infant rats were advanced lesions of nodular hyperplasia observed as reported by Himsworth and Glynn.[17b] Probably these rats did not live long enough for fibrosis to develop to the point of causing severe distortion and nodularity of the liver. Microscopically, areas of liver with parenchymatous cells completely dead were seen alternating with areas of normal liver parenchyma (fig. 1). In places the necrosed cells could be seen being phagocytosed by polymorphonuclear leukocytes, although areas of necrosis without any leukocyte reaction were not uncommon in such livers. Fatty infiltration of the liver cells was not seen to any impressive degree. In the livers of the animals which had survived the initial illness, a noninflammatory fibroblastic reaction was noted in the necrotic areas. Although the special stains did not show any appreciable increase of fibrous tissue, the quantitative determination revealed an increase of collagen ranging from 0.5 to 0.8 per cent.

Another lesion encountered was the enormous dilatation of sinusoids (fig. 2) with no definite areas of necrosis or fibrosis. The liver cells bordering the dilated sinuses were flattened and had a finely granular and often vacuolated cytoplasm which did not stain for fat. Some cells around the portal tracts contained fat droplets, which were mostly small and numerous, although an occasional large globule could be seen occupying the whole cell and pushing the nucleus to one side. Special stains did not reveal an increase of reticulum but showed the reticulum condensed along the liver cell cords.

Livers of Rats Weighing 100 *Gm. or More.*—The changes observed in the livers of the older animals were different. These livers showed heavy fatty infiltration, represented mostly by single large fat globules, in almost all the liver cells (fig. 3). The sinusoids associated with the distended cells were almost obliterated. At this and later stages, staining with phloxine-methylene blue did not reveal Mallory's hyaline bodies, supposed to be characteristic of alcoholic cirrhosis. In animals living longer the condensing of reticulum fibers along the liver cell cords was seen. This was followed by the stretching of the fine reticulum fibers that circumscribe the lobules. The liver cells at the periphery of the lobules showed degenerative changes. The cytoplasm was granular and vacuolated, and the nuclei were pyknotic. These vacuolated cells have been described by Mallory[109] and Connor[55a] as characteristic of active cirrhosis.

Among those rats maintained for a longer time on the diet an increasing condensation of reticulum occurred around the lobules as well as inside the lobules along the liver cell cords (fig. 4). Fibrous tissue was being laid down along the large portal tracts. In some regions the bile ducts appeared proliferated and dilated. At this stage there was a definite decrease in the amount of fat in the liver cells, and whatever there was occurred in the form of small discrete droplets. None of the livers with outstanding fatty infiltration showed any necrotic areas, although predominantly necrotic changes, associated with minimal fatty infiltration, similar to changes in the livers of the younger rats, were also seen in some adult animals.

In the early stages no microscopic evidence of hepatic damage was seen. The sections stained with the trichrome stain did not reveal definite fibrosis. The stain for reticulum showed increasing condensation of the fibers. The most reliable method was the quantitative determination of fibrous tissue by the method of Lowry and associates.[178] The average normal quantity ranged up to 0.4 per cent of the wet weight of the liver, as determined in 8 normal rats. Histologically, none of these showed increased reticulum. In early cases in which microscopic examination showed only condensation and increase of the reticulum, the quantitative

determination gave figures up to 0.9 per cent. In only 1 rat, in which the amount of fibrous tissue was 1.2 per cent, did the microscopic examination give definite evidence of early cirrhosis in the form of fibroblastic activity and increased periportal fibrosis.

Table 2 shows the amounts of fibrous tissue in pathologic livers.

The adult rats thus showed hepatic changes comparable to early portal cirrhosis of man, though a few revealed only focal necrosis. None had the advanced cirrhosis characterized by irregularity of surface externally and fibrosis and disorganization of the lobular pattern microscopically. Only 1 rat had ascites, which was minimal, and none showed jaundice. There was no definite line of demarcation between the types of pathologic change produced by the casein-deficient and the yeast-deficient diet except that the animals restricted to the latter diet showed lesions earlier.

TABLE 2.—*Correlation of Microscopic and Chemical Evidence of Fibrosis in Rats' Livers*

Rat	Microscopic Observation	Fibrous Tissue, Per Cent of Wet Weight of Liver
1	Increased reticulum?	0.5
2	Increased reticulum	0.8
3	Increased reticulum	0.75
4	Increased reticulum	0.9
5	Increased reticulum	0.85
6	Increased reticulum and increased periportal fibrosis	1.2

COMMENT

The lesions seen in the rats on the high carbohydrate, low protein diet in the present experiment are different in some respects from the changes reported by other workers, especially Himsworth and Glynn[17b] and Gillman and associates.[16] The diet used was the synthetic diet nearest to the natural high carbohydrate and low protein meals eaten by orthodox Hindus, whose staple diet is wheat, rice or maize. The children also eat the same foods in the form of gruel or porridge.

Changes produced in infant were different from those produced in adult rats. In the former the outstanding lesion was focal or diffuse necrosis followed by noninflammatory fibroblastic activity. That acute necrosis is followed by fibrosis was shown by the increase of fibrous tissue as determined by quantitative estimation. This finding is consistent with the changes Himsworth and Glynn[17b] observed in the livers of rats subsisting on their high carbohydrate, protein-deficient diets except that none of my rats showed extensive scarring and macroscopic nodular hyperplasia. It may be that the rats in the present series did not survive long enough for extensive fibrosis to develop. None of the livers showed fatty changes.

Himsworth and Glynn[17b] made a similar observation and stated that "fatty infiltration of liver is not a necessary antecedent of massive necrosis, but may, on the contrary, serve as a protection against its development."

Some of the infant rats also showed dilatation of sinusoids, with associated flattening of liver cells, and diffuse enlargement of the lobule, but none of them showed atrophy or disappearance of the affected lobules as reported by Gillman and co-workers.[16] Hemorrhage was also absent, though at times sinusoidal distention with cellular atrophy was mistaken for it.

In the adult rats the liver cells' accumulation of fat seemed to be the obvious reaction to the high carbohydrate, low protein diet. In some of the animals patches of focal necrosis developed similar to those in the infant rats; the same animals had only minimal fatty infiltration. This is identical with the observation of Himsworth and Glynn[17b] that hepatic necrosis developed with minimal fatty infiltration in rats fed protein-deficient diet.

However, a good percentage showed extensive fatty infiltration of the liver, almost all the cells being filled with single large fat droplets. The livers of these rats resembled those reported by György and Goldblatt,[181] Chaikoff, Connor and Biskind,[49] Blumberg and Grady[51] and Gillman and associates.[16] Variations were seen in the further development of the disease process from this stage onward. Some of the livers did not show any increase of reticulum either microscopically or chemically. However, they showed an increase of weight due to fatty infiltration, and when the amount of fibrous tissue determined quantitatively was calculated relative to the normal weight of livers, these livers could be classified as precirrhotic (Warren and Wahi[179]). Other livers showed microscopic signs of incipient cirrhosis—decrease of fat, condensation and thickening of perilobular and intralobular reticulum, increase of periportal connective tissue with small round cell infiltration, dilatation and proliferation of bile ducts, and quantitative increase of fibrous tissue content. The type of cirrhosis observed by Blumberg and Grady,[51] Chaikoff and associates,[50] and Connor[55a] in depancreatized dogs, by Connor[55b] in human beings suffering from alcoholism and in patients with diabetes and by Gillman and associates[16] in rats was preceded by fatty infiltration of liver cells of long standing, but there was no hemorrhage or necrosis. Himsworth and Glynn[17b] have recently reported that diffuse hepatic fibrosis developed in rats restricted to a high fat diet. This was preceded by fatty infiltration of the liver cells. In the presence of severe protein deficiency they observed the development of hemorrhagic necrosis affecting the entire liver. If the animal succumbed, they felt that this was similar to the acute yellow atrophy observed in man; if it survived, it showed

181. György and Goldblatt (footnotes 59*a* and *c*).

nodular hyperplasia of the liver comparable to the healed yellow atrophy in man.

The left lobe of the liver was found to be more involved than the right, and in certain cases the pathologic change was confined to the left lobe. This confirms the similar observations of Himsworth and Glynn.[17b] Their explanation for it seems the most acceptable. They ascribed it to the fact that in the portal vein the blood coming from the superior mesenteric vein tends to the right and so passes up the right branch of the portal vein to the right lobe of the liver. The blood from the splenic and inferior mesenteric veins keeps mainly to the left and reaches the left lobe of the liver. This difference has been demonstrated also in dogs on injection of emulsified fat (Bartlett and co-workers[182]), india ink (Copher and Dick[183]) and radioactive phosphorus (Hahn, Donald and Grier[184]). When the material was injected into the superior mesenteric vein, it was carried to the right lobe, and when it was injected into the inferior mesenteric or the splenic vein, it was carried to the left lobe. A similar tendency has been noted by Gillman.[14]

Thus the right lobe of the liver is supplied through the superior mesenteric vein with blood predominantly from the small intestine and receives thereby the products of the latter's digestion, which contain great amounts of those constituents of protein required to prevent massive necrosis. When the supply of these essential constituents is insufficient, the right lobe gets most of it and escapes necrotic changes. In the left lobe, receiving blood from the large bowel, which is poor in protein constituents, necrosis develops.

From the available evidence there seems to be little doubt that fatty livers can be produced by deficient diets, whether these contain a high percentage of fat or not. In the present series, as well as in those reported by Gillman and associates,[16] high carbohydrate, protein deficient diets have produced extensive fatty infiltration. Such livers may undergo necrosis and postnecrotic scarring, or they may undergo cirrhosis. György and Goldblatt[58] made a careful study of the possibility that exogenous or endogenous toxins may cause the hepatic damage observed in their animals. They observed that damage of the liver occurred only under specific dietetic conditions and came to the conclusion that if toxins were responsible they exerted their effects only on animals having a particular dietetic background. Their animals first showed diffuse fatty changes of the liver, and thereafter some other factor, either exogenous or en-

182. Bartlett, F. K.; Corper, H. J., and Long, E. R.: Am. J. Physiol. **35**:36, 1914.

183. Copher, G. H., and Dick, B. M.: Arch. Surg. **17**:408, 1928.

184. Hahn, P. F.; Donald, W. D., and Grier, R. C., Jr.: Am. J. Physiol. **143**:105, 1945.

dogenous, induced hemorrhagic necrosis and finally a condition which they considered cirrhosis but which probably was postnecrotic scarring and nodular hyperplasia. In the experiments described by Gillman and associates,[16] the livers became diffusely fatty, and thereafter there was great diversity in the sequence of events in the development of the pathologic process in the different lobes of the same liver.

In the present series both these changes were seen either individually or concomitantly in rats fed a diet high in carbohydrate and low in protein. The obvious indication is that with the same diet and under the same conditions the animals may get focal or diffuse necrosis with or without postnecrotic scarring, fatty infiltration alone or followed by incipient cirrhosis, and, finally, a combination of postnecrotic scarring and portal cirrhosis. What is it, then, which decides what type of lesion develops? Is it the length of time which the animal survives on the deficient diet? As Himsworth and Glynn[17b] noted, "the animals least adroit in obtaining food would die of necrosis, those more adroit would survive to develop diffuse fibrosis." However, this does not seem to be the whole story, for on the basis of the present experiments, as well as on the basis of the work of Gillman and associates,[16] it can be said that fatty liver is an essential precursor of cirrhosis, although even when of long standing the fatty infiltration does not necessarily terminate in cirrhosis. This leads one to the conclusion that the injury of the liver cells implied in fatty infiltration does not necessarily lead to fibroblastic reaction. This is in accordance with the observations of Gillman and associates,[16] who noted the disappearance of the whole of the trabeculae and the lobules without any evidence of fibrous tissue reaction and concluded that nodular hyperplasia and cirrhosis are manifestations of stimuli exciting proliferative activity of both the liver cells and the connective tissue.

Mallory[109] also pointed out that fibroblasts do not proliferate when liver cells alone are destroyed. Lucke[185] reported similar pathologic observations in regard to epidemic hepatitis, in which there was no formation of collagen though there was extensive destruction of parenchyma. The independence of the hepatic parenchyma and the connective tissue with respect to injury was also noted by Daft, Sebrell and Lillie.[165e] The question again arises as to the role of a possible toxin, endogenous or exogenous, acting on a protein-depleted liver. The absence of lipotropic amino acids leads to fatty infiltration, but the proliferation of fibrous tissue requires an independent stimulus. Is the proliferation of fibroblasts in alcoholic cirrhosis due, as Mallory[109] suggested, to injury caused

mechanically by cells of exudative origin stretching the connective tissue, or is it due to a toxin whose nature is yet to be determined?

It is interesting to compare these experimental observations with those on the livers of infants with cirrhosis in India, where the histologic development of cirrhosis is more on an infectious basis. Broad bands of fibrous tissue, patches of necrosis, cellular infiltration, complete disorganization of the liver pattern and absence of fatty changes are outstanding in the microscopic observations. The changes are more of the nature of postnecrotic scarring leading ultimately to nodular hyperplasia, rather than portal cirrhosis. Clinically, infantile cirrhosis in India is often a familial disease. The illness is usually ushered in by fever and other symptoms of acute infection. These facts make one wonder whether infection of some sort does not play a precipitating role, especially in the light of Rao's[134] report that he obtained a culture of B. coli in one of his cases. He described polymorphonuclear infiltration of the periportal areas as a constant finding on microscopic examination. It may be that in the familial instances the cause is a virus infecting the mother's genital tract, the infant contracting the infection during birth. This suggestion is along the lines of recent work of Budding[186] in which he isolated a virus identical with the virus of infantile diarrhea from the adult female genital tract. He observed that "the probability that the virus may be acquired, transmitted, and maintained venereally in adults and thus serve as the original source of epidemics of diarrhea of the new-born, must be entertained." Nevertheless, the absence of protective proteins in the undernourished vegetarian infant plays an important role in that it prepares the liver to be finally knocked out by a precipitating factor like infection.

SUMMARY AND CONCLUSIONS

Rats weighing approximately 50 Gm. and fed a diet high in carbohydrate and low in protein show hepatic damage, which expresses itself as necrosis of the parenchyma. This may end in postnecrotic scarring if the animal lives long enough.

Animals weighing over 100 Gm. are more resistant to the effect of this deficient diet and live longer. Their livers show varieties of lesions like focal or diffuse necrosis, fatty infiltration and incipient cirrhosis.

In the development of cirrhosis of the liver there are two stages; the first consists of diffuse fatty infiltration of the liver cells, and the second, of fibrous tissue proliferation and regeneration of liver cells.

185. Lucke, B.: Am. J. Path. **20**:471, 1944.

186. Budding, G. J.: South. M. J. **39**:382, 1946.

Cirrhosis of the liver is not a necessary sequel to injury of the liver cells, and fatty infiltration does not necessarily terminate in cirrhosis.

It is possible that some noxious agent like a toxin, bacterium or a virus, especially in infantile cirrhosis, acts as a precipitating factor.

The quantitative determination of fibrous tissue has been found more useful in determining the presence of cirrhotic changes in early stages than the microscopic examination of stained sections.

CONGENITAL MALFORMATION OF VERTEBRAE (HEMIVERTEBRAE) WITH APLASIA OF CORRESPONDING RIBS, ASSOCIATED WITH A LATERAL MENINGOMYELOCELE

A Report of a Case

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CONGENITAL absence of one or more ribs accompanied or not accompanied by hemivertebrae is relatively rare. When, in addition, such a congenital defect is accompanied by a lateral meningomyelocele, it is truly a rarity. These defects may occur with or without other congenital anomalies.

Several papers[1] published before the advent of roentgenography reported cases of absence of one or more ribs in which the diagnosis was made purely on physical examination. Three cases,[2] however, had the benefit of autopsy. The first roentgenographically proved case of congenital absence of a rib was reported in 1899 by Freund.[3] In 1944 Cohn[4] reviewed all the cases of congenital absence of one or more ribs in the literature and classified them into three groups: (1) those in which one rib was missing unilaterally or bilaterally[5]; (2) those wherein

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1. (*a*) Lallemand, 1826, cited by Kienböck.[6b] (*b*) Packard, J. H.: Proc. Path. Soc. Philadelphia **2**:121, 1867. (*c*) Gage, H.: Tr. Am. Orthop. A. **2**:233, 1889. (*d*) Sabrazès: Rev. de méd. **14**:1010, 1894. (*e*) Thomson, J.: Teratologia **2**:1, 1895. (*f*) Murray: Tr. Clin. Soc. London **29**:252, 1896; cited by Smith.[6e]

2. Ardouin, P., and Kirmisson, E.: Rev. d'ortop. **8**:104, 1897. Gripat, H.: Bull. Soc. anat. de Paris **47**:124, 1874; cited by Smith.[6e] Vrolick, cited by Kienböck.[6b]

3. Freund, W.: Jahrb. f. Kinderh. **49**:349, 1899.

4. Cohn, B. N. E.: Am. J. Roentgenol. **52**:494, 1944.

5. (*a*) Allen, I.: Canad. M.A.J. **28**:69, 1933. (*b*) Anderson, W. W., and Cathcart, D. F.: Arch. Pediat. **49**:827, 1932. (*c*) Fabris, S.: Pediatria **34**:1310, 1926. (*d*) Gladstone, R. J.: J. Anat. & Physiol. **46**:220, 1911. (*e*) Hadda, S.: Ztschr. f. orthop. Chir. **31**:176, 1913. (*f*) Hatch, H. S., and Plume, C. A.: J. A. M. A. **99**:1254, 1932. (*g*) Lieberknecht, A.: Beitr. z. klin. Chir. **51**:89, 1906. (*h*) Pels-Leusden: Chir. Kongr.-Verhandl. **72**:191; cited by Hadda.[5e] (*i*) Putti, V.: Fortschr. a. d. Geb. d. Röntgenstrahlen **14**:285, 1909; **15**:65 and 243, 1910. (*j*) Sever, J. W.: Boston M. & S. J. **186**:799, 1922.

two or more consecutive ribs were lacking on the same side[6]; (3) a miscellaneous group which does not fit either of the foregoing classifications.[7] He presented cases which fitted each classification and pointed out that congenital absence of a rib is commonly associated with presence of a hemivertebra.

An intrathoracic meningocele presenting a round shadow in roentgenograms was reported by Pohl[8] in 1934. In 1940 Ameuille, Willmoth and Kudelski[9] reported finding an intrathoracic mass in a 48 year old patient who complained of thoracic pain. A surgical procedure revealed the mass to be a lateral meningocele with intrapleural development arising from a lateral defect in the eighth and ninth thoracic vertebrae. Arnt[10] reported a simple case of hemivertebrae in 1946.

REPORT OF CASE

The patient was a full term white boy who died the second day following delivery. There was no familial history of congenital defects, nor was there a history of contagious diseases occurring during the mother's pregnancy. Examination revealed a white boy with a chest grossly deformed as a result of absence of the first through the ninth right ribs. The tenth, eleventh and twelfth right ribs appeared to be fused posteriorly. In the right posterior wall of the chest there was a large orange-shaped mass which was soft, cystic and could be transilluminated. A thoracic scoliosis to the left was present. There was a clubbed right foot, also a congenital dislocation of the right hip.

Autopsy.—Postmortem examination revealed complete absence of the right halves of the first through the ninth thoracic vertebrae and absence of their transverse processes. The corresponding right ribs were also absent. The tenth, eleventh and twelfth right ribs were present but were fused posteriorly. Extending laterally to the right from the spinal canal through a defect in the neural arches of the first through the ninth thoracic vertebrae was a pedicle 2.0 by 1.5 cm. in size, which was continuous with the meninges. The pedicle also included a portion of spinal cord. Attached laterally to this pedicle was a large, cystic structure measuring 6.0 by 5.5 by 5.0 cm. and containing a clear fluid. It possessed no communication with either the arachnoid or the subdural spaces. However, a communicating channel was present in the pedicle between the cystic structure and the central canal of the spinal cord just superior to the pedicle. It barely admitted a probe. The central canal of the spinal cord superior to this lumen was greatly dilated, measuring 1.0 cm. in diameter. The surrounding neural tissue was greatly thinned

6. (*a*) Erkes, F.: Deutsche Ztschr. f. Chir. **114**:239, 1912. (*b*) Kienböck, R.: Fortschr. a. d. Geb. d. Röntgenstrahlen **13**:269, 1909. (*c*) Rees, H. L.: Lancet **1**:916, 1930. (*d*) Riether, G.: Wien. klin. Wchnschr. **23**:306, 1910. (*e*) Smith, C.: J.A.M.A. **60**:895, 1913. (*f*) Steel, W. D.: Brit. M. J.: **2**:16, 1939. (*g*) Hadda.[5e] (*h*) Putti.[5i]

7. Gotzky, F., and Weihe, F.: Fortschr. a. d. Geb. d. Röntgenstrahlen **21**: 408, 1914. Joachimstahl, G.: Ztschr. f. orthop. chir. **25**:18, 1910. Putti.[5i] Sever[5j]

8. Pohl, R.: Röntgenpraxis **5**:747, 1934.

9. Ameuille, P.; Willmoth, P., and Kudelski, C.: Bull. et mém. Soc. méd. d. hôp. de Paris **56**:608, 1940.

10. Arnt, F.: Röntgenpraxis **14**:384, 1942.

out and measured only 0.1 to 0.2 cm. in thickness. The cystic mass was covered on its anterior surface by parietal pleura (figs. 1 and 2). A clubfoot was present on the right. The head of the left femur was displaced posteriorly and inferiorly to a position beneath the gluteal muscles. The right lung was smaller than the left and had no middle lobe. A small azygos lobe measuring 0.7 by 0.4 by 0.3 cm. was present on the anterior medial aspect of the upper lobe of the right lung. The heart revealed a widely patent foramen ovale measuring 0.5 cm. in diameter.

Fig. 1.—Roentgenogram showing congenital hemivertebrae of the first through the ninth thoracic vertebrae associated with aplasia of the corresponding right ribs. Note the fusion of the tenth, eleventh and twelfth right ribs.

The ductus Botalli was widely patent. The right renal artery, renal vein, kidney and ureter were absent. The left kidney was of average size and shape and weighed 15 Gm. Its pyramids were somewhat blunted, and the corresponding calices were ballooned out. The pelvis was somewhat dilated. The left ureter was markedly dilated and tortuous. It measured 1.3 cm. in circumference. Its wall was thickened. Its ureterovesical orifice was widely patent. The right testicle was present in the abdominal cavity; the left was present in the inguinal canal. The left adrenal gland was of normal size and shape and weighed 2.8 Gm. The right adrenal gland was less than half the size of the left and weighed only 1.0 Gm.

COMMENT

Hemivertebra and the associated aplasia of the corresponding rib can be explained on an embryologic basis. From the ventromesial face of each somite a group of mesenchymal cells arise which collectively are called a sclerotome. These mesenchymal cells migrate from each side toward the midline, becoming aggregated about the notochord. It is from these masses of cells that the elements of the vertebral column and the ribs arise.

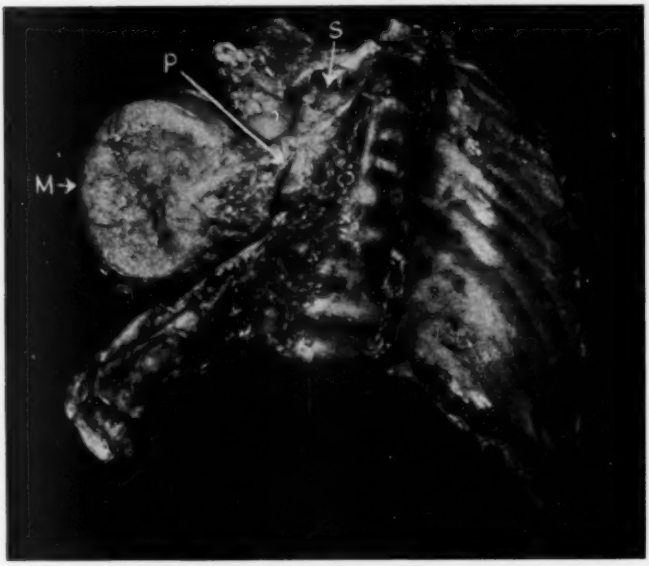

Fig. 2.—Dissection of the thorax showing the absence of the right half of the first through the ninth vertebrae and their corresponding right ribs. *M* indicates the meningomyelocele; *P*, the pedicle; *S*, the syringocele.

The first significant change that occurs in these primordial masses is the clustering of sclerotomal cells, derived in part from each of two adjacent somites, into groups which are located opposite the intervals between the myotomes. Each of these cell clusters forms the primordium of the centrum of a vertebra. Once formed, they rapidly become more dense and definitely circumscribed (fig. 3). Shortly after the formation of the centrum, paired mesenchymal concentrations extended dorsally and laterally from the centrum becoming the primordia of the neural arches and ribs (fig. 4).

The blastemal stage is the stage in which the earliest parts of the skeleton become recognizable and is rapidly followed by the cartilage

stage. The conversion to cartilage begins in the blastemal masses, first in the region of the centrum, following which centers appear in each neural arch and costal process. These centers spread rapidly and fuse

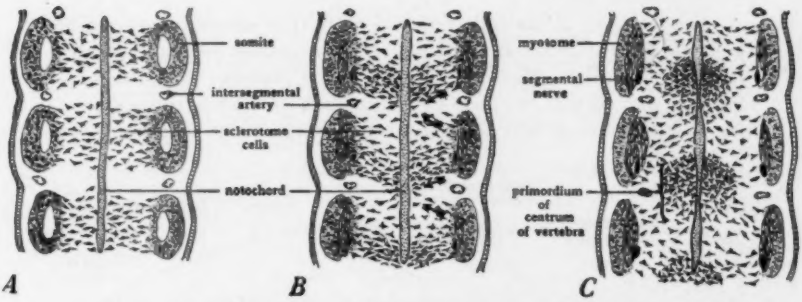

Fig. 3.—Mesenchymal cells arising from the ventromesial faces of the somites (in *A*), migrating toward the midline represented by the notochord (in *B*) and becoming aggregated about the notochord (in *C*). Each cell cluster in *C* forms the primordium of the centrum of a vertebra. (The illustrations shown in figures 3, 4 and 5 are taken from Bradley M. Patten's book "Human Embryology," published in 1946 by the Blakiston Company, Philadelphia.)

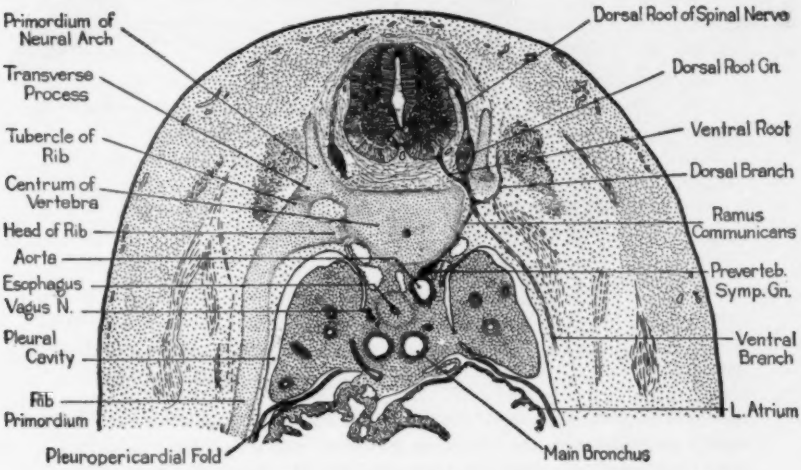

Fig. 4.—Primordia of the neural arch and transverse process of the vertebra and primordia of the rib—all being developed out of the centrum of the vertebra.

until the entire mass is involved. The cartilaginous miniature of the vertebra thus formed is first a single piece showing no lines of demarcation where the original centers of cartilage formation became confluent.

Neither does it reveal the separate parts of which it will consist after the cartilage has been replaced by bone. By the time ossification begins, the rib cartilages have become separated from the vertebrae themselves, remaining in one piece.

The locations of the endochondrial ossification centers appearing in a vertebral cartilage are shown in figure 5. The median ossification center forms the centrum. The centers in the neural processes extend dorsally forming laminas that complete the neural arch. A prolongation of these same centers beyond their point of junction, dorsal to the centrum, forms

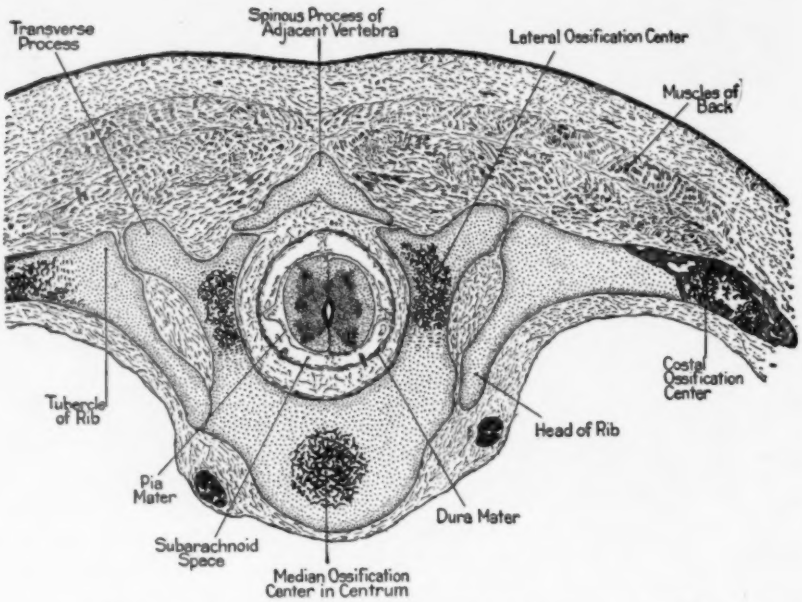

Fig. 5.—Further development of the vertebra, with centers of ossification indicated.

the spinous process. Lateral extensions of the centers appearing in the neural processes form the transverse processes with which the ribs articulate. The neural processes also extend ventrally fusing with those in the centrums.

The shafts of the ribs are formed by extensions of their primary ossification centers. Secondary epiphysial centers appear in the tubercle and in the head of the rib after birth. During the period of growth they remain separated from the shafts of the ribs with persistence of cartilage

plates. The secondary epiphysial centers fuse with the shaft when the skeleton acquires its adult dimensions.[11]

Thus the relationship between the primordium of a rib and the corresponding vertebra is developed from the embryologic point of view. It is seen, therefore, that anything interfering with the development and growth of the primordium of a vertebra may simultaneously be reflected in the growth and development of the transverse process, the rib, the neural arch and even the spinous process—consequently, the frequently associated aplasia of a rib and hemivertebra.

Early writers felt that defects of the development of ribs were due to pressure of an arm against the thoracic cage secondary to a lack of amniotic fluid.[12] They felt that the arm occupied a hernial defect in the thoracic wall. However, the relationship between aplasia of a rib and hemivertebra tends to rule out this theory. Stockard[13] in 1921, as a result of his work on the developmental changes occurring in the periods of both determinate and indeterminate cleavage resulting from outside influences, such as changes in hydrogen ion concentration, moisture, oxygen supply and temperature, in the eggs of Fundulus, postulated a more widely accepted theory. He felt that in the development of every organ or part there is a critical stage characterized by rapid cell multiplication during which it is dominant over nearby organs. If during this critical period an environmental change acts adversely on the dominant organ, it may lose its dominance to another organ; this results in an imperfectly formed organ. Thus not only must the organ originate from a definite primordium, but it must arise at the appropriate time if it is to assume its normal development and growth. This theory appears most tenable in explaining a congenital hemivertebra and the associated aplasia of the corresponding rib. Simultaneously the adverse stimulus responsible for the formation of a hemivertebra may act on other developing organs during their critical period to produce concomitant congenital defects. The defect of the neural arches present in this case allowed the meninges to herniate into the posterior wall of the chest with resultant formation of a meningomyelocele.

SUMMARY

A case of a lateral meningomyelocele with absence of the right halves of the first through the ninth thoracic vertebrae and the associated ribs is presented, together with roentgenograms and autopsy observations. The embryonal development of the vertebral body and its corresponding ribs is discussed, as is the theory of the genesis of these defects.

11. Patten, B. M.: Human Embryology, Philadelphia, The Blakiston Company, 1946.

12. Ritter and Eppinger, cited by Hadda.[5e] Kienböck.[6b] Thomson.[1d]

13. Stockard, C. R.: Am. J. Anat. **28**:115, 1921.

EXPERIMENTAL LIPOSARCOMA

Characteristics of Growth Under Low and Under High Caloric Intake

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THE PERTINENT problems of fat tissue new growth are the same as those which obscure the embryogenesis of normal fat tissue. A chemical difference of the lipoma fat and the fat of the normal fat depots has been proposed but no difference of sufficient magnitude has been revealed by chemical methods. An analysis of lipoma fat by Jaeckle[1] showed a remarkably close resemblance to normal subcutaneous human fat. The fatty masses of adiposis dolorosa were also found to be quite similar to normal fat by Edsall.[1] Failing adequate explanation on a structural or a chemical basis, Wells[2] has suggested that tumor fat tissue differs from normal subcutaneous fat because of deficiency or abnormality of the enzymes of fat metabolism. This theory was supported by the conclusion of Kastle and Leavenhart[1] that fat storage is dependent on the presence of the enzyme lipase, which acts reversibly either to split fat into fatty acids and glycerin or to synthesize fat from these diffusible constituents as they are provided by the blood. The absence of lipase in the tumor fat might explain its inavailability to the host fat tissue; it does not explain, however, how storage of fat occurs in the tumor.

Nerve supply and hormone control have been given consideration. Boeke's[3] demonstration that isolated sympathetic nerve fibers are present in individual cells and that denervated fat tissue deposits contain more fat than normal deposits has some bearing on the conception that abnormal nerve control may lead to abnormal storage and metabolism of fat. The observation of Beznak and Hasch[4] that there is an increase in weight of the fat deposits on the sympathecto-

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1. Cited by Wells.[2]
2. Wells, G.: Arch. Int. Med. **10**:297, 1912.
3. Boeke, J.: Ztschr. f. mikr.-anat. Forsch. **33**:233, 1933.
4. Beznak, A. B., and Hasch, Z.: Quart. J. Exper. Physiol. **27**:1, 1937.

mized side and that fat persists longer at the site of intervention in emaciation further emphasizes the influence of the sympathetic supply on trophism and growth of fat tissue. The same is true of hormone control, so much emphasized that any further mention would be repetition. Along this line is Geschickter's report[5] that xantholipomatous proliferations were produced in subcutaneous tissue by injecting chorionic gonadotropin extracted from pregnancy urine.

Although the evidence at hand is not sufficient to allow the conclusion that caloric restriction may be used to affect tumor growth, controlled experiments have shown that caloric intake as well as the percentage and the composition of ingested fat and of certain members of the vitamin B complex can influence carcinogenesis (King[6]). If this is the case for all tumor growths, regardless of their origin and structure, as seems likely, it must be more true for the fat tissue new growths, since they arise from cellular elements constantly subject to a variety of complex chemical processes, synthesis, interconversion, degradation, all comprehensively indicated by the expression "molecular regeneration."

PURPOSE OF INVESTIGATION

The main point of the present investigation was to study the influence of caloric restriction, on one side, and of a high caloric intake, on the other, in the development and progress of an experimentally produced lipid growth.

Although conclusions as to normal cell origin cannot properly be drawn from the study of rapidly multiplying tumor cells, still it was thought that by following the patterns of growth closely some valuable information might be obtained about the growth of normal and cancerous fat tissue.

MATERIAL AND METHODS

White mice of identical breed, male and female indifferently, from 6 to 8 weeks old, and weighing from 12 to 15 Gm., were used as experimental animals. As carcinogenic agent 1, 2, 5, 6-dibenzanthracene was used. The basic diet fed to the animals consisted of the usual mice biscuits containing not less than 14.50 per cent crude protein, 2.05 per cent crude fat, 18 per cent crude fiber and 44 per cent nitrogen-free extract. Ingredients were oats, corn meal, soy bean oil meal, corn germ meal, alfalfa meal, wheat gray middlings, molasses, riboflavin supplement, 1.5 per cent calcium carbonate and 0.5 per cent iodized salt. The group of animals whose caloric intake was to be restricted received daily one third of the ration given the control group. To the basic diet of the group of animals that were to receive high caloric diet a slice of bacon was added daily.

A striking loss of weight in the animals of the first group and an equally striking increase of weight in the animals of the second group, little affected by

5. Geschickter, C. F.: Am. J. Cancer **21**:617, 1934.
6. King, C. G.: Ann. New York Acad. Sc. **44**:3, 1947.

the development of the tumor, were evidence of the effects of the diets. This was further shown at the postmortem examinations by the depleted aspects of the fat depots in one group of animals and by their repleted appearance in the other group.

As it was felt that the composition (melting point, length of fatty acid chains) and therefore the rate of absorption of the fatty solvent carrying the carcinogenic hydrocarbon might have some bearing on the incidence and progress of the tumor, for some of the animals the carcinogen to be injected was dissolved in corn oil, and for some other animals the same carcinogen was dissolved in emulsified pork fat.

With all groups of animals a 5 per cent solution of the carcinogen was used; 0.3 cc. of the compound was injected into the subcutaneous fat of each axillary fossa, each inguinal region and the interscapular gland.[7] This distribution was suggested by the known abundance of fat tissue in these locations.

Over the entire experimental period, which lasted nine months, all the animals were systematically examined every six days. Some of the animals were killed at the first appearance of the tumor, others were killed after increasing intervals, and in still others the tumor was allowed to follow its natural course.

The 48 mice used in the experiment were divided into the following four groups: (1) animals receiving 1, 2, 5, 6-dibenzanthracene in corn oil solution—low caloric intake (14 animals); (2) those receiving 1, 2, 5 6-dibenzanthracene in corn oil solution—high caloric intake (14 animals); (3) those receiving 1, 2, 5, 6-dibenzanthracene in pork fat solution—standard diet (10 animals), and (4) those receiving 1, 2, 5, 6-dibenzanthracene in corn oil solution—standard diet (10 animals). Group 4 was the control group.

RESULTS

Structural Characteristics of the Produced New Growth.—Regardless of the difference of caloric intakes and of vehicles of the carcinogen, the gross and the structural characteristics of the growth were invariably the same in the different groups of animals. The gross appearance of each tumor was that of a poorly defined mass, moderately firm in consistency. The color varied from area to area, with an alternating of yellowish gray and grayish pink areas, which found an explanation in the complexity of the cellular patterns as revealed by the microscopic study. In no instance was evidence found that the tumor had metastasized to any of the internal organs.

Besides the structural characteristics, the marked tendency of the tumor to infiltrate locally and the successful transplanting of three different tumors into two additional series, each consisting of 2 animals, clearly showed the aggressive tendencies of the growth; 0.5 cc. of a suspension of the tumor (1 to 5 in saline solution) injected deeply into the subcutaneous tissue of the groin was productive in all but 1 of a fairly

7. The interscapular gland is a peculiar mass of tissue composed of an interlacing of vascular lymphoid tissue and of fat cells. A number of students have accredited to it a lipid storage function corresponding to that of hibernating glands of insectivora and bats.

rapidly growing tumor that exactly reproduced both grossly and microscopically the characteristics of the original tumor. Recession of the growth did not take place in any case, and in a few instances the tumor

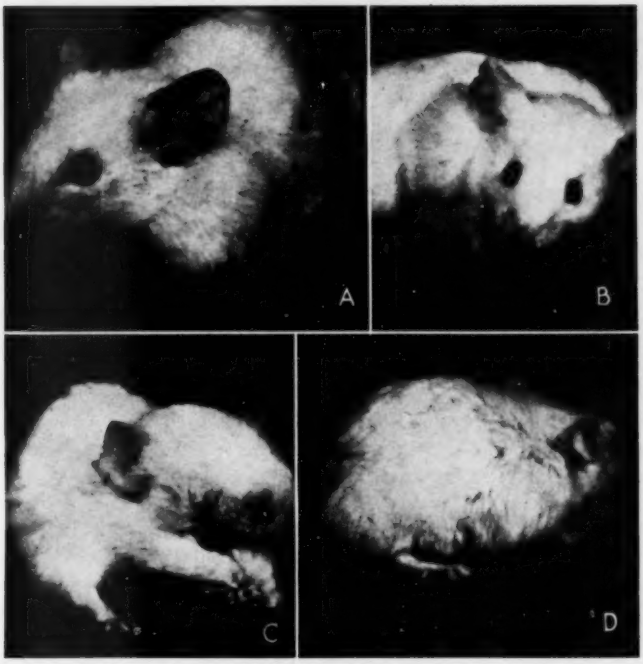

Fig. 1.—*A*, mouse 45, one hundred and forty days after injection of 1, 2, 5, 6-dibenzanthracene, displaying a mass at the left axillary region. The mouse was given the carcinogen in corn oil with the standard diet.

B, mouse 35, one hundred and thirty-eight days after injection of 1, 2, 5, 6-dibenzanthracene, showing two large new growths, one at the right inguinal region and the other at the right axilla. The mouse received carcinogen in pork fat with the standard diet.

C, mouse 3, one hundred and fifty days after injection of 1, 2, 5, 6-dibenzanthracene, revealing a large mass in the interscapular region extending over the scalp and forehead. The mouse was given the carcinogen in corn oil with a low caloric diet.

D, mouse 16, one hunded and forty-five days after injection of 1, 2, 5, 6-dibenzanthracene, showing tumor involvement of the right axillary and inguinal regions. The mouse was given the carcinogen in corn oil with a high caloric diet.

showed a tendency to ulcerate at the surface; however, in order to detect the progressive developmental phases of the growth, many animals were killed before the tumor had reached full development, and therefore it

cannot be ruled out that this complication might have occurred more frequently if the animals had survived longer.

The structure of the tumor consisted mainly of a loose-textured framework of well vascularized fibrous tissue in which a variety of cells was contained, widely spaced in some areas and closely packed in others. The most common cell type was represented by spindle cells, thickly interlaced. They resembled basically the cells of medullary fibrosarcoma, but the cellular endings tended to be blunt instead of pointed, and the cytoplasm was more acidophilic than in the ordinary fibrosarcoma cell. The nuclei were often hyperchromatic and the nucleoli prominent. Mitosis was at the rate of 1 per high power field. Staining for fat droplets revealed none in these spindle cells. The intercellular substance had a few delicate interlaced argentaffin fibers in the meshes of which was contained an amorphous eosinophilic material which could not be stained with mucicarmine.

Fat cells, either sparse or grouped together in alveolar arrangement, were present in this fibrosarcomatous tissue. In some areas the lipid structures prevailed; in some others the fibrosarcomatous elements were predominant. The fat cells ranged in shape from oval to round or polyhedral, and closely resembled the steatoblast or embryonal fat cell of rodents. In the adult fat cells the nuclei occupied the usual peripheral position; in the more immature fat cells the nuclei were either peripherally or more centrally placed. Some of the nuclei were small and vesicular, others large and deeply stained, occupying from one third to one half the diameter of the cell. An occasional nucleus contained a single large nucleolar structure, almost as large as the entire nucleus. The cellular cytoplasm had a foamy appearance and contained a delicate network of granules which enclosed fat globules. The latter ranged from small and dustlike to large granules occupying a good portion of the cellular cytoplasm.

In the midst of these fat cells, irregularly stellate cells, often fused together in syncytial masses, and very large cells containing bizarre-shaped nuclei were not uncommon. These large cells varied in size depending on the degree to which the eosinophilic and finely granular cytoplasm was distended by fat globules. At first glance these giant cells seemed to contain several nuclei; however, on more careful examination each cell was found to possess a single nucleus with a varied number of lobes connected by narrow bands of nuclear substance. In their intimate cytologic characteristics these large cells closely resembled the small granular immature fat cells, and often transitional patterns could be recognized between the two cell types.

Another element in the growth that deserves special mention was a round cell which in many respects resembled a large plasma cell. The

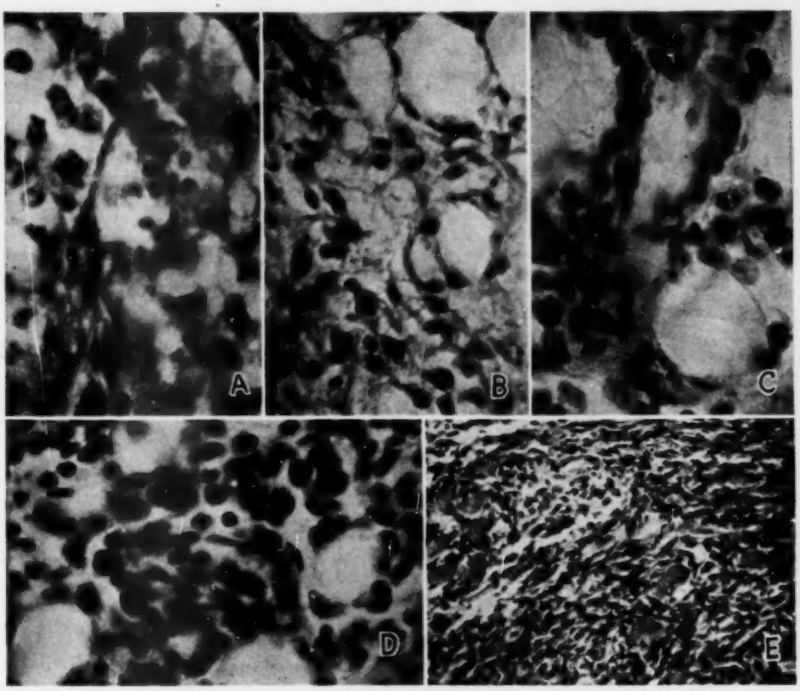

Fig. 2.—*A*, early histiocytic mobilization in the fat tissue septums. The tissue was removed from a nodule 0.3 cm. across in the right inguinal region of a mouse ninety days after injection of the carcinogen. This mouse belonged to group 1, fed a low caloric diet. Photomicrograph, ocular 5, objective 40, Zeiss.

B, pleomorphic proliferation of mesenchymal cells in the fat tissue septums. The geat variability of cellular forms exhibited is regarded as an expression of the multiple developmental potentialities of the "dormant" undifferentiated mesenchymal cells of the fat lobule which under carcinogenic stimulation differentiated into a variety of connective tissue cells. The tissue was removed from a nodule 0.5 cm. across in the left axillary region of a mouse ninety-eight days after injection of the carcinogen. This mouse belonged to group 2, fed a high caloric diet. Photomicrograph, ocular 5, objective 40, Zeiss.

C, diffuse infiltration of the fat tissue septums by lymphocytoid cells and by cells resembling large plasma cells. Cells with two nuclei are present. The tissue was taken from a nodule 0.4 cm. across in the left inguinal region of a mouse one hundred and three days after injection of the carcinogen. The mouse belonged to group 4, fed the standard diet. Photomicrograph, ocular, 5 objective 40, Zeiss.

D, predominance, in the growth, of cells resembling plasma cells with patterns suggesting transitional stages in the development of lipoblastic cells. The tissue was removed from a nodule 0.3 cm. across in the right axillary region of a mouse one hundred and nine days after injection of the carcinogen. The mouse belonged to group 2, fed a high caloric diet. Photomicrograph, ocular 5, objective 40, Zeiss.

E, thickly interlaced spindle cells, resembling basically the cells of medullary fibrosarcoma, infiltrating the muscle bundles of the thigh. The tissue was taken from a full grown tumor of the right groin, 3 cm. across, occurring in a mouse six months after injection of the carcinogen. This mouse belonged to group 1, fed a low caloric diet. Photomicrograph, ocular 10, objective 10, Zeiss.

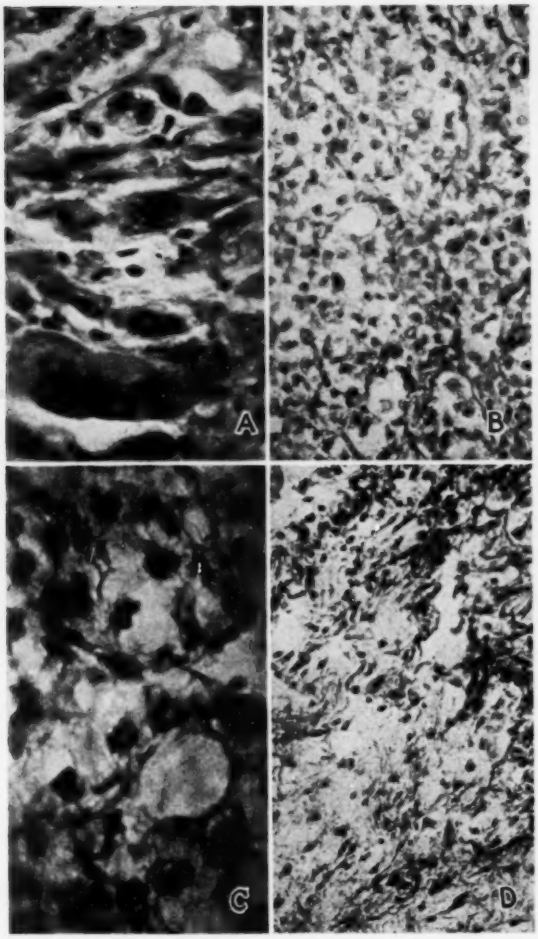

Fig. 3.—*A*, giant cells containing a single nucleus with a varied number of lobes connected by narrow bands of nuclear substance. In their intimate cytologic characteristics these large cells resemble the small immature granular fat cells; transitional patterns could often be recognized between the two cell types. The tissue was taken from the same tumor as that shown in figure 2*E*. Photomicrograph, ocular 10, oil immersion, Zeiss.

B, low power view of a full grown tumor from the interscapular region showing predominance of lipid cells. The tissue was removed from a mouse of group 2, fed a high caloric diet, five months after injection of the carcinogen. Photomicrograph, ocular 10, objective 10, Zeiss.

(Legend continued on opposite page)

nucleus, displaced to the periphery, contained several nucleoli and fine purplish violet chromatin granules in a background of pinkinsh karyoplasm (parachromatin). Against the identifying of this cell with the typical plasma cell of the connective tissue was, however, the less sharp cellular outline, the absence of a clear perinuclear area and the less pronounced cytoplasmic basophilia.

These cells were not conspicuous in the full grown tumor mass, as they appeared to be outnumbered by the other cellular elements, but were instead a predominant feature during the early developmental stages of the growth; they could be detected in the animals killed at the first appearance of the tumor. Distinct transitional patterns noted between these cells and cells with characteristics of histiocytes, on one side, and cells with features of lipoblasts, on the other, led to the impression that they represented an intermediate stage in the development of the lipoid cells. The first step in their differentiating along the line of the lipid-storing cells was found to occur in an inversion of the nucleocytoplasmic ratio, to the advantage of the cytoplasm, in a tendency of the nucleus to become centralized, and in an increase in the size of the cell. The lipid storage function of these cells was already apparent at this stage of development, as shown by the presence of small cytoplasmic sudanophilic droplets. A spongy appearance of the cytoplasm, its becoming amphophilic or eosinophilic, and a greater amount of fat droplets appeared to characterize the following stage of development, and when a number of these cells lay close together, they became polyhedral in shape, because of reciprocal compression, and showed a tendency to arrange themselves in clusters. The active part taken by them in the growth was shown by the frequency with which cells with two nuclei could be recognized among the proliferating cells.

Incidence of New Growth Under Low and Under High Caloric Intake.—The incidence of the growth in the two groups of animals, those fed a low caloric diet (group 1) and those fed a diet of high caloric value (group 2), is shown in table 1. Cancer developed in 7 of the 14 animals of group 1; a slightly higher incidence of growth—10 of 14—was shown by the animals of group 2, but there is less difference between the two groups of animals when it is considered that 3 animals

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C, high power view of tissue from the same tumor as that represented in *B*. That the newly formed cells closely resemble the steatoblast or embryonal fat cell of the rodent is apparent. Photomicrograph, ocular 10, objective 40, Zeiss.

D, section from another field of the same tumor from which those reproduced in *B* and *C* were obtained, showing a loose-textured framework of myxomatous connective tissue with stellate cells irregularly scattered. Photomicrograph, ocular 7, objective 10, Zeiss.

of group 1 died at the beginning of the experiment in an extremely emaciated condition.

The development of the growth at one site of inoculation of the carcinogen did not prevent its development at another site of injection of the same agent; this led to a total of fifteen tumors in group 1 and seventeen in group 2. Growths in symmetric regions—axillas, groins—occurred in 2 animals, once in each group.

TABLE 1.—*Comparison of Results Obtained with a Carcinogen in a Group of Mice Fed a Low and a Group Fed a High Caloric Diet*

Animal	Site of Injection: Interscapular Region	Groin: R	Groin: L	Axilla: R	Axilla: L
	Treatment: Carcinogen in Corn Oil—Low Caloric Diet				
1	—	—	—	—	—
2	+	—	+	+	—
3	+	—	—	—	—
4	Died early in the experiment				
5	+	—	+	+	—
6	—	—	—	—	—
7	Died early in the experiment				
8	—	+	+	+	—
9	—	—	—	—	—
10	Died early in the experiment				
11	—	—	—	+	—
12	—	—	—	—	—
13	—	+	—	—	+
14	—	—	+	+	—
	Treatment: Carcinogen in Corn Oil—High Caloric Diet				
15	+	—	—	+	—
16	—	+	—	+	—
17	—	—	—	—	—
18	—	—	—	—	—
19	+	—	+	—	+
20	—	—	—	—	—
21	—	—	+	—	—
22	—	—	—	+	—
23	—	+	—	+	—
24	+	—	—	+	+
25	—	—	+	—	—
26	—	—	—	—	—
27	—	—	+	—	—
28	—	—	+	—	—

Note: + indicates growth; — indicates no growth.

No significant difference of the two groups of animals was noticed in regard to the time of first appearance of the tumors. Growth was first noticed in the right groin of an animal of group 1 ninety days after the injection of the carcinogen, and six days later a growth was apparent in the left groin of an animal of group 2. From the ninetieth to the one hundred and eighteenth day the growths started to appear in the other animals. Once the tumor had started in any animal it enlarged rapidly

so as to reach a diameter of as much as 3 cm. in the course of four to six weeks; no differences were noticed in the rate of growth of the tumor in the two groups of animals.

From the foregoing observations the conclusion can be drawn that differences of caloric intake and the ensuing depletion or increase of fat in the depots as realized by the procedures used in the present experiment did not seem to influence in any apparent way the anaplastic growth which resulted from carcinogenic stimulation.

TABLE 2.—*Comparison of Results Obtained with a Carcinogen Dissolved in Pork Fat and in Corn Oil*

	Site of Injection				
Animal	Interscapular Region	Groin R	Groin L	Axilla R	Axilla L
	Treatment: Carcinogen in Pork Fat—Standard Diet				
29	—	—	—	—	—
30	—	+	—	—	+
31	—	—	—	—	—
32	—	—	—	—	—
33	+	—	—	—	—
34	—	—	—	—	—
35	—	+	—	+	—
36	—	—	—	—	—
37	—	—	—	+	—
38	—	—	—	—	—
	Treatment: Carcinogen in Corn Oil—Standard Diet				
39	+	—	—	+	—
40	—	—	+	—	—
41	—	+	—	—	—
42	+	—	+	—	—
43	—	—	—	—	—
44	+	+	—	+	—
45	—	—	—	—	+
46	+	—	+	+	—
47	—	—	—	—	+
48	—	—	—	—	—

+ indicates growth; — indicates no growth.

The Fatty Solvent Carrying the Carcinogen as a Factor in the Incidence of the Growth.—A comparison of the results observed in the animals receiving the carcinogen in corn oil solution and those receiving the same amount of the carcinogen dissolved in emulsified pork fat—both fed the standard well balanced diet—shows some difference in the incidence of the tumor growth (table 2). Carcinoma developed in 4 of the animals receiving the carcinogen dissolved in animal fat and in 8 of those receiving the carcinogen in corn oil solution, with a total of six tumor growths in the former and fourteen in the latter. Further indication that the vegetable oil solvent might favor prompter action of the carcinogen was found in the earlier appearance of the growth both

in this group of animals and in the similarly treated animals in which development of the growth was studied under low and high caloric diets. Whereas in the animals receiving the carcinogen dissolved in corn oil of the present series the tumor growth made its first appearance between the ninety-fifth and one hundred and twentieth day, in the animals receiving the same amount of carcinogen dissolved in pork fat the first evidence of incipient growth was noticed at the one hundred and twenty-ninth day after inoculation, and within the successive thirty-seven days the tumor made its appearance in only 3 additional animals of the group. Once the development of the tumor had started, no differences were noticed in the rate of growth between the two groups of animals, the tumor reaching a size ranging to 3 cm. in a period of four to six weeks. The possibility of eliciting multiple tumor growths in the same animal, already shown by the previous experimental series, was confirmed in this group of animals, with a higher incidence in the corn oil group. The gross appearance of the tumor and its intimate cytologic characteristics were the same in the two groups of animals, and successful transplants were obtained from both.

Although the small number of animals used in the experiment limits the conclusion, a point worthy of further investigation seems to emerge from these observations: that the composition and the rate of absorption of the fatty solvent carrying the carcinogen may have some bearing on the incidence and on the time of appearance of the elicited new growth.

COMMENT

In the face of the known ability of benzpyrene, dibenzanthracene and other related chemicals to induce cancerous change in many different types of tissues, the production of cancerous growths in the present series by injecting the carcinogen in the midst of fat tissue is not surprising.

It is generally accepted that the fat-forming cells are found in embryonal life in two different forms: (1) widely spaced stellate or spindle-shaped cells, which gradually become rounded as they accumulate lipoid material, immersed in a mucoid intercellular substance; (2) lipid cell groups in glandlike lobules of a moruloid or mulberry appearance in the absence of any mucoid intercellular substance. Both forms are reproduced in the cancerous growths arising from lipid cells, hence Ewing's[8] proposal that these growths be classified as of two types: adult liposarcoma and myxoliposarcoma. The adult type is composed of rounded or polygonal lipoblasts of varying sizes and of adult fat cells without myxomatous or fibroblastic tissue taking any active part in the growth. The

8. Ewing, J.: Arch. Surg. **31**:507, 1935.

myxoliposarcoma consists of an admixture of adult fat cells and fat-laden fibrocytes embedded in a fibrosarcomatous tissue. The latter was the structure displayed by the tumor produced in the present series.

Among the tumors obtained by comparable experimental procedures, the closest resemblance is found in the liposarcoma produced by Haagensen and Krehbiel[9] in mice and guinea pigs by repeatedly injecting 1, 2-benzpyrene into the subcutaneous fat tissue. In the growth produced by Haagensen and Krehbiel the striking pattern was that of an atypical proliferation of cells resembling steatoblasts and polymorphonuclear giant cells, which in turn could be compared to the giant multinucleated lipoblasts described by Murray[10] in a transplantable liposarcoma of the guinea pig. The main difference between Haagensen and Krehbiel's tumor and the one presented here consists, however, in the more active part played in the latter by fibroblastic elements; hence it is classified as embryonal sarcoma.

Regardless of the difference of caloric intake and of the vehicle of the carcinogen, the greater incidence of new growths (see tables 1 and 2) obtained in the animals of the present series following a single injection of the carcinogen as compared with the rarity with which new growths of a similar type were induced by Haagensen and Krehbiel employing repeated injections of a closely related carcinogen may find an explanation in the higher concentration of the carcinogen and in its having been introduced more directly into the fat deposits in the present series.

Haagensen and Krehbiel[9] concluded their observations by suggesting that the tumor growth had originated from "adult fat cells as the result of their long continued chemical irritation and stimulation." A similar conclusion is not warranted in the case of the growth under consideration. No mitotic figures or signs of direct cell division, and no other patterns suggesting proliferation of adult fat cells were noticed in any of the growths of the present series.

Whether or not the adipose cells are capable of multiplying once they have reached their full maturity is still a point of controversy. This possibility was admitted by Kölliker[11] but was denied more recently by Wassermann,[12] who advanced the opinion that fat cell proliferation occurs through a dedifferentiation by which the adult fat cell is returned to the original pluripotent embryonal reticulum cell capable of giving rise not only to new fat cells but to a variety of connective tissue cells, hematic cells included.

9. Haagensen, C. D., and Krehbiel, O. F.: Am. J. Cancer **27**:474, 1936.
10. Murray, J. A.: J. Path. & Bact. **20**:260, 1916.
11. Kölliker, A.: Anat. Anz. **1**:206, 1886.
12. Wassermann, F.: Ztschr. f. Zellforsch. u. mikr. Anat. **3**:235, 1926.

Without ruling out the possibility that proliferation of fat cells may occur under certain conditions through a subdivision of the nuclei of adult fat cells, as indicated by Kölliker, or through retrogression of the adult fat cells, as thought by Wassermann, personal observations on atrophic fat, on fat tissue under inflammatory stimulation and on spontaneous cancerous fat tissue new growths have led to concurrence in the impression that fat cell proliferation, both cancerous and noncancerous, takes place according to the same plan as that by which in embryonal development undifferentiated mesenchymal cells are brought to mature into fat cells. This is accomplished by the revival of the dormant histiocytic cell of the fat tissue, which under the influence of a variety of stimuli rapidly differentiates into a fat cell, going through the same cellular metamorphosis that the mature fat cell experienced in its development.

Evidence supporting this conception was found also in the early developmental stages of the tumor under consideration, which regardless of the localization seemed invariably to start from a multicentric proliferation of the undifferentiated mesenchymal cells embedded in the fat tissue lobule. Cells lymphocytoid in appearance and larger cells which in many respects resembled the so-called reticular plasma cell of the bone marrow prevailed in these early stages. In this connection it is worth recollecting that cells similar to those under consideration are mentioned in a number of descriptions of the development of embryonal fat tissue. Basing his conclusions on the finding of cells resembling plasma cells in embryonal fat tissue, Waldeyer[13] expressed the view that the wandering plasma cell of the connective tissue can be mobilized for the function of storing fat. Bodritzky[14] advanced a similar opinion; and in the first unitarian conception bearing on the origin of fat cells, Poljakoff[15] concluded that Waldeyer's "plasma cell," Ranvier's "fat cell," Ehrmann's "mulberry cell" and the "fat cell" of the subcutaneous tissue are merely modifications of an embryonal connective tissue cell. More recently Geschickter[5] in reviewing a large series of lipoid tumors stressed the presence in cancerous growths of a cell type resembling a "plasma cell or fetal cartilage" and pointed out the possibility that it might represent the forerunner of the larger foam cell.

The intimate admixture, in the present growth, of cells resembling fibroblasts and of lipoid cells in various stages of development again raises the question, debated so many times, of the genetic relationship of the two cell types.

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13. Waldeyer: Arch. f. mikr. Anat. **11**:176, 1875.
14. Bodritzky: Centralbl. f. d. med. Wissensch. **23**:753, 1885.
15. Poljakoff: Arch. f. mikr. Anat. **32**:122, 1888.

Commenting on the origin of the fat cells in a case of lipomyxosarcoma, Jacobson[16] interpreted the clustering of mucin about fat droplets in the cells of the growth as evidence of the close relationship of the fat cell and the fibroblast.

Against the assumption that imbibition of fat is a potential function common to all connective tissue cells, fibroblasts included, is, however, the fact of common knowledge that in some parts of the body fat tissue new growths are extremely uncommon and that no matter how great the general adiposity can be, fat does not become deposited in the connective tissue. If the conception that the cell of the fibrous connective tissue may store fat is to be accepted, one has therefore to conceive the existence of two kinds of fibroblasts, one linked to the lipid metabolism and the other independent from it. Along this line is the conclusion of Maximow[17] that the specialized fat cell is entirely distinct from the fibroblast and that both in embryonal and in adult life fat tissue cells are formed from undifferentiated mesenchymal cells situated about blood vessels.

The description by Murray and Stout[18] of a liposarcoma growing "in vitro" includes cellular elements with the cytoplasmic but not with the nuclear characteristics of a fibroblast. Burkhardt[19] also, in cultures of bone marrow of adult guinea pigs, has shown that the capacity developed by the adipose tissue for fat storage is not coincident with the loss of other mesenchymal potentialities; while containing large vacuoles of fat, these cells were still capable of locomotion and phagocytosis, and even of undergoing cell subdivision; at times they appeared as ameboid, wandering cells, at other times as fibroblast-like cells, and occasionally they displayed the appearance of multinucleated giant cells.

The gamut of cells described by Burkhardt[19] and by Murray and Stout[18] is run by the growth described here. The great variability of cellular forms which it exhibited might be regarded as an expression of the multiple developmental potentialities of the undifferentiated mesenchymal cell of the fat lobule, which under carcinogenic stimulation differentiated into a variety of connective tissue cells, of which the lipoblasts were merely an offshoot with distinct characteristics.

SUMMARY

Another tumor type is added to the several forms of sarcoma which have been induced by means of carcinogenic hydrocarbons. 1, 2, 5, 6-di-

16. Jacobson, V. C.: J. Cancer Research **6**:109, 1921.
17. Maximow, B.: Textbook of Histology, Philadelphia, W. B. Saunders Company, Philadelphia, 1934.
18. Murray, M. R., and Stout, A. P.: Am. J. Path. **19**:751, 1943.
19. Burkhardt, L.: Arch. f. exper. Zellforsch. **16**:187, 1934.

benzanthracene injected in the midst of fat tissue deposits of white mice was productive in a large number of animals of a transplantable new growth provided with the characteristics of an embryonal cell liposarcoma. The great variability of cellular forms which it exhibited is regarded as an expression of the multiple developmental potentialities of the undifferentiated mesenchymal cell of the fat lobule which under carcinogenic stimulation differentiated into a variety of connective tissue cells, of which the lipoblasts were merely an offshoot with distinct characteristics. From observations on early new growths evidence was obtained that lipid cell cancerous proliferation takes place according to the same gamut of cellular changes by which in embryonal development undifferentiated mesenchymal cells are brought to mature into fat cells.

Differences in caloric intake and the ensuing depletion or increase of fat in the depots did not seem to influence in any apparent way the incidence and progress of the growth.

The development of a growth at one site of inoculation of the carcinogen did not prevent the development of another growth at another site of injection of the same agent. This resulted in a number of instances in a picture grossly resembling a multicentric lipoblastosis.

SACCULATED MECKEL'S DIVERTICULUM INCORPORATED IN THE WALL OF THE ASCENDING COLON

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PITTSBURGH

MECKEL'S diverticulum, estimated to occur in approximately 2 per cent of persons, is recognized to vary greatly in size, shape, position, attachments and structure.

In the case to be described, a peculiar, and perhaps unique, structure and appearance of Meckel's diverticulum prevented recognition of the diverticulum at operation.

A white youth 17 years old was admitted to the hospital with the typical symptoms and signs of partial or complete intestinal obstruction of four days' duration. He was severely dehydrated. After an attempt to restore normal water balance, an incision was made through the right rectus abdominis muscle and the peritoneum, with the patient under ether anesthesia. A distended and congested small bowel was encountered. The terminal part of the ileum was twisted and tightly wrapped around what appeared to be an anomalous portion of bowel 4 inches (10 cm.) long and ½ inch (about 1 cm.) in diameter, producing complete obstruction. The anomalous bowel had no mesentery and extended from a point on the terminal part of the ileum about 75 cm. above the cecum to another point on the ascending colon just above where the ileum enters into the cecum. It appeared to be a communication between the ileum and the ascending colon. The twisted ileum was loosened, and the anomalous portion of intestine was resected from the ileum and the cecum by electrocautery. The openings were inverted by purse string sutures. The twisted small bowel was dusky in color but appeared viable after release of the obstruction. The abdominal incision was closed tightly in layers. The patient passed gas and some feces by rectum after the operation, and at first his condition seemed improved. However, his abdomen remained distended, and a moderate fever was present. He died six days after the operation, apparently from generalized peritonitis.

An autopsy was performed one and one-half hours after death. In the lower part of the abdomen there was a healing recent incision of the right rectus abdominis, 15 cm. long. The peritoneal cavity contained 2,000 cc. of cloudy, yellowish gray, foul-smelling fluid, and all peritoneal surfaces were covered by thick, yellow, fibrinous exudate. The small intestine was considerably distended with fluid and gas throughout. The site of resection on the ileum had ruptured and presented an opening 0.5 cm. in diameter. This was 72 cm. above the ileocecal junction. The cecum, the appendix and the ileum had their normal relationships. On first inspection, the ascending colon exhibited its usual external appearance

From the Institute of Pathology and the Landon Surgical Clinic of the Western Pennsylvania Hospital.

except for the peritoneal exudate and the presence of a 2 mm.-sized opening on the serosal surface, just above the ileum, where the anomalous portion of intestine had been resected. On dissecting this area, however, it was found that the opening did not enter the ascending colon but instead entered a blind pouch 4 cm. long, 4 cm. wide and 3 cm. thick, intimately incorporated in the wall of the ascending colon and covered by peritoneum (fig. 1). The pouch contained thick,

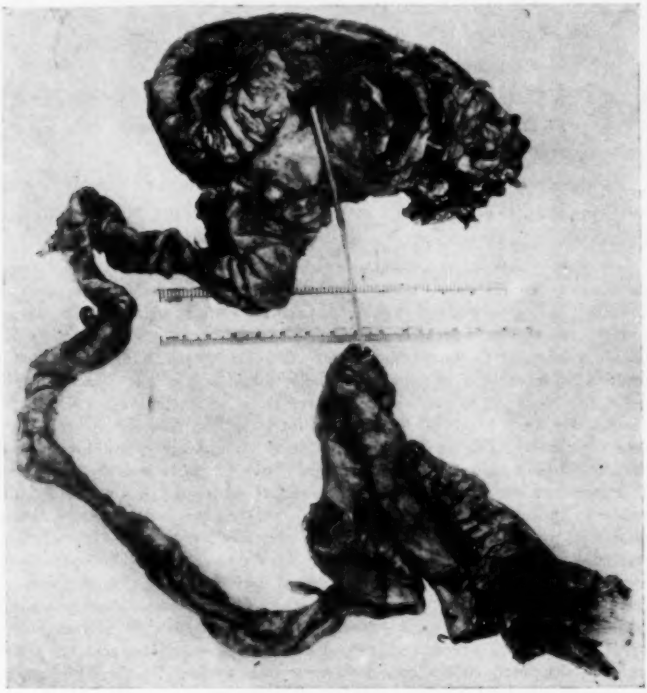

Fig. 1.—Photograph showing the cecum and the ileum with a probe representing the excised portion of the diverticulum. The lateral aspect of the cecum is at the top of the picture. The pouch of the diverticulum has been opened to the right of the probe.

yellow, turbid fluid and the lining resembled intestinal mucosa. The lumen of the pouch was separated from the lumen of the ascending colon by a septum 1.5 cm. thick. The floor of the pouch was 1 cm. above the ileocecal valve (fig. 2).

A schematic representation is seen in figure 3.

Microscopic sections of the pouch showed a mucosal lining suggestive of ileum, which was in great part necrotic, extensively ulcerated and covered with fibrinous exudate. Indications of peritoneal remnants were seen in elastic laminas present in the connective tissue between the muscularis of the pouch and that of

the ascending colon, on the one hand, and the muscularis of the ileum, where the latter entered the cecum, on the other.

No other abnormalities were found at the autopsy.

The elastic laminas identified between the muscularis of the pouch and surrounding structures indicate that the distal end of this Meckel's diverticulum had been completely invested by peritoneum but had be-

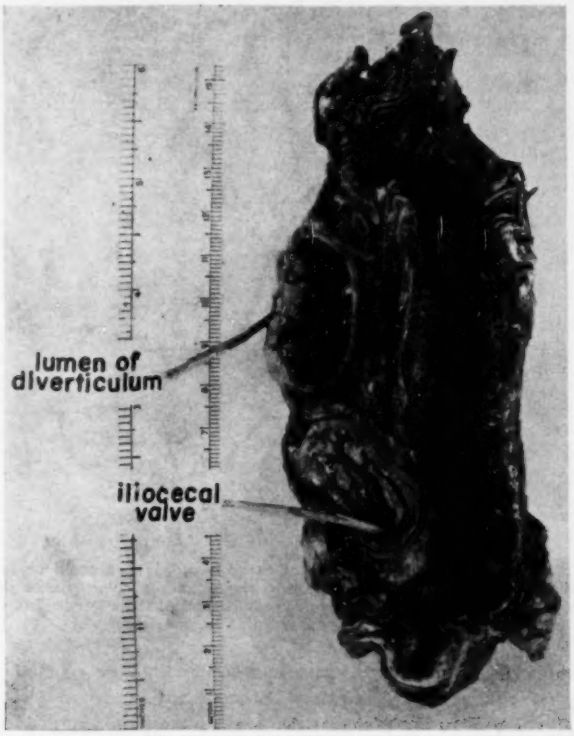

Fig. 2.—Photograph showing a cross section of the cecum and the ascending colon from a posterior point of view. The medial side of the specimen is to the left and the cecum is at the bottom.

come adherent to the wall of the ascending colon. It either was originally dilated at the distal end or else the dilation occurred after it became incorporated in the wall of the ascending colon. The latter mechanism seems more likely, since partial obstruction may have been caused by the sharp angle at which the proximal part of the diverticulum joined it.

The distal end of the diverticulum was so well incorporated in the wall of the ascending colon that the exact nature of the anomaly was not recognized at operation. It was thought to be an anomalous portion of bowel connecting the lumen of the ileum with that of the ascending colon—a kind of "double terminal ileum." In fact, even after it was removed at autopsy, its true nature was determined only on subsequent dissection of the specimen.

A review of the literature discloses only 1 case[1] in which a somewhat similar complication of Meckel's diverticulum was described, although

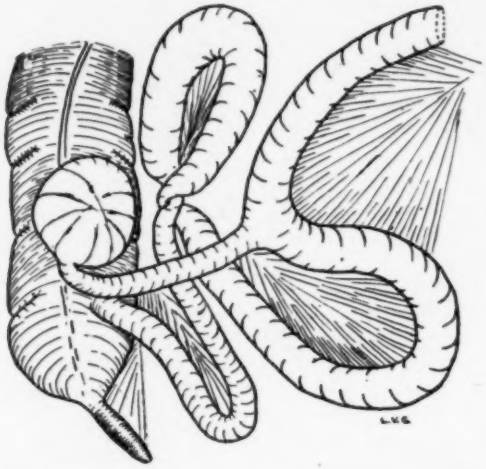

Fig. 3.—Reconstruction drawing of the condition encountered at operation, with the medial aspect of the cecum and the ascending colon turned anteriorly to expose the diverticulum. The dilated terminal end of the diverticulum is drawn in prominently, although it was not discovered at operation.

the manner in which the diverticulum adhered to the cecum was evidently not that of being intimately incorporated in the wall of the ascending colon as in the present case. Also no mention was made of sacculation of the distal end of the diverticulum. This patient recovered after reduction of the volvulus and removal of the diverticulum and the appendix.

Complications incident to Meckel's diverticula are relatively rare in view of the known frequency of these diverticula. Intestinal obstruction is the most common cause of such complications. In many of the cases it has been due to the presence of a fibrous cord extending from the tip

1. Miller, R. H., and Wallace, R. H.: Ann. Surg. **98**:713, 1933.

of the diverticulum to some other part of the abdomen, usually the umbilicus or the mesentery and abdominal wall, less frequently to other structures.

SUMMARY

A case in which the saccular dilated distal end of Meckel's diverticulum was incorporated in the wall of the ascending colon is reported. This anomaly was observed in a 17 year old white youth who died of generalized peritonitis following resection of the proximal, unincorporated part of the diverticulum. A volvulus of the terminal part of the ileum was associated with the anomaly. The colonic wall's incorporation of the sacculated distal end of the diverticulum was so complete that this complication was not recognized at operation or on first inspection at autopsy.

HEMANGIOSARCOMA OF THE SPLEEN

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PRIMARY splenic neoplasms, because of their variety and rarity, are pathologic curiosities. In 1945 Bostwick[1] provided a workable classification, based on cell types, and brought the total of reported splenic tumors of all varieties to 161.

A review of the literature with reference to the neoplasm reported here revealed that there is a small group of primary splenic tumors which are characterized by well defined vascular channels, by areas of sarcomatous aspect histologically, and usually by metastases. This group was recognized by Wright[2] but since these tumors appear under several headings, it seems worth while to reassemble them and to report an additional case.

REPORT OF A CASE

C. K., a 78 year old white man, was admitted to St. Luke's Hospital for the fourth time June 15, 1945.

His past history revealed yellow fever in 1874, malaria in 1892, a venereal wart in 1900, a "generalized eruption" in 1901 and typhoid fever in 1905. He was admitted in 1935 for a direct inguinal hernia and prostatic hyperplasia. Examination revealed an enlarged prostate gland, an inguinal hernia and a hydrocele. Dilated abdominal veins were noted. A hemogram and the urine were within normal limits. Serologic tests for syphilis revealed none. A roentgenogram of the chest and an electrocardiogram disclosed no abnormalities. Transurethral prostatic resection and bilateral vasotomy were performed. The patient was readmitted in January 1936 for repair of the inguinal hernia; this was followed by uneventful convalescence. In December 1940 he was admitted subsequent to irrigation of the right antrum. At that time crepitus was noted in the infraorbital tissues. This resolved in twelve hours, and the patient was discharged without further study.

From 1940 to 1945 he complained of intermittent pain occurring in the lower part of the back and in the legs. It was associated with increasing fleeting dull pains in all bones and with severe pain in the left upper quadrant of the abdomen. It was described as a dull ache which was occasionally punctuated by sharper pain radiating from the back around both sides of the abdomen above the umbilicus. Weakness and fatigability were prominent symptoms for one month prior to admission.

From the Department of Pathology, St. Luke's Hospital.

1. Bostick, W. L.: Am. J. Path. **21**:1143, 1945.
2. Wright, A. W.: Am. J. Path. **4**:507, 1928.

June 15, 1945, he underwent a sudden syncope and was admitted for observation. Examination revealed a malnourished, pale white man with an icteroid tinge of the skin. The tongue showed no papillary atrophy. There were decreased breath sounds at the apex of the right lung. Marked tenderness of the left costovertebral angle and the left upper quadrant of the abdomen (spleen) was found. The spleen could not be palpated. Moderate prostatic hyperplasia was noted, and also a few small internal and external hemorrhoids. No neurologic changes were observed.

Examination of the urine was noncontributory. The hemogram at admission was as follows: hemoglobin, 5.6 Gm. per hundred cubic centimeters; red blood cells, 1,900,000, and white blood cells, 9,700, per cubic millimeter; polymorphonuclears, 86 per cent; lymphocytes, 12 per cent; monocytes, 2 per cent; platelets, 70,000 per cubic millimeter; reticulocytes 0.1 of 1 per cent; hematocrit, 22; color index, 1; volume index, 1.2; mean corpuscular hemoglobin, 29.9 micromicrograms; mean corpuscular volume, 110 cubic microns. Considerable basophilic stippling was noted in smears; marked anisocytosis and poikilocytosis and rare nucleated red cells were observed while the differential count was being made. Wassermann and Kahn tests for syphilis were negative. Chemical examination of the blood showed: urea nitrogen, 19.4 mg. per hundred cubic centimeters; icterus index, 8; acid phosphatase, 1.8 Gutman units; erythrocyte sedimentation rate, 13 mm. per hour (Westergren). The stool showed no blood. Gastric analysis revealed no free hydrochloric acid after histamine stimulation. Roentgen examination of the chest revealed a mass of homogeneous soft tissue filling the apex of the right lung and extending down to the third rib posteriorly. This appeared to produce destruction of the posterior aspect of the second rib. Pleural exudate filled the costophrenic sulcus on both sides. Barium sulfate studies of the colon revealed no abnormalities.

Admission diagnoses of heat stroke, prostatic hyperplasia and possible pernicious anemia were made. Following roentgen examination of the chest, a diagnosis of generalized carcinomatosis of undetermined origin was entertained.

The patient was given 15 units of concentrated liver on June 21 and on three successive days thereafter. A reticulocyte response of 20.1 per cent was noted on the fifth day of liver therapy. The temperature remained normal, and the patient appeared to be doing well until the thirteenth hospital day, when he died suddenly without obvious clinical reason.

Autopsy twenty-four hours after death showed a well developed but thin 78 year old white man. Seven hundred cubic centimeters of serous fluid was found in the right pleural cavity and 400 cc. of similar fluid in the left. The lungs were increased in weight and showed marked hypostatic congestion. There was a small fibrotic area at the right apex. The heart weighed 575 Gm. The myocardium was pale and flabby. The papillary muscles were calcified. There was atherosclerosis of the large vessels. In the abdominal cavity, in the left upper quadrant, was a large mass replacing the spleen and displacing the stomach and the left kidney. The liver (1,700 Gm.) showed numerous dark bluish red soft nodules 3 to 5 mm. in diameter beneath the capsule; "nutmeg" congestion was prominent and small dark bluish red nodules were scattered throughout all lobes. The gallbladder and the bile ducts were normal. Similar small dark reddish blue nodules were scattered over the parietal and to a lesser extent over the visceral peritoneum. The pancreas, the adrenal glands, the esophagus, the stomach and the duodenum were normal. Meckel's diverticulum was found in the ileum, and the jejunum contained numerous diverticula between the leaves of the mesentery. The appendix was present. The colon was normal except that the splenic flexure was displaced downward by the mass in the upper quadrant.

The spleen weighed 1,700 Gm. and measured 19 by 15 by 12 cm. It was adherent to the diaphragm and to the abdominal wall. The capsule was covered by soft fibrous adhesions. The spleen was moderately firm and dark red. The sectioned surfaces showed deep red lobulated areas surrounding areas of hemorrhage and infarction. No normal-appearing splenic tissue remained. The splenic artery and vein and the portal vein were normal. Numerous small, deep red retroperitoneal lymph nodes were noted.

The right kidney was a hydronephrotic cyst with the ureter stenosed at its junction with the pelvis. The left kidney weighed 175 Gm. The bladder was dis-

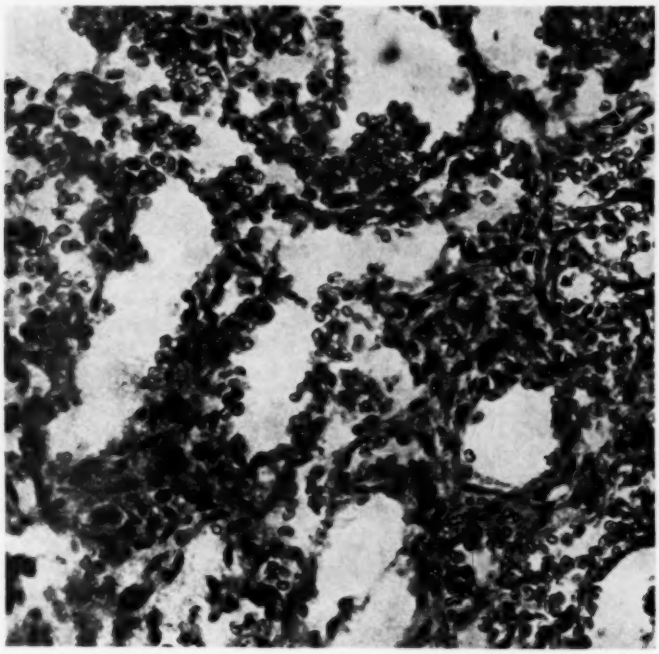

Fig. 1.—Hemangiosarcoma of the spleen showing elliptic channels and lined with atypical endothelial cells containing blood cells; × 375.

tended with clear urine, and scars were noted near the trigone. There was prostatic hyperplasia of both lateral lobes. All vertebral bodies contained dark hemorrhagic areas with bony rarefaction. The second right rid and the eighth left rib both contained hemorrhagic masses.

Microscopic examination of the lungs revealed an area of fibrosis and scarring with bronchiectases and lymphoid infiltration. The heart showed muscular atrophy. There was chronic passive congestion of the liver. There were numerous small areas of hemorrhage surrounded by a network of small capillary spaces, the majority containing red blood cells. These spaces were lined by spindle-shaped

endothelial cells which appeared larger than the endothelial cells of the hepatic sinusoids and showed considerable nuclear irregularity. These capillaries were thin-walled and closely bound to a loose connective tissue stroma. Surrounding the hemorrhagic area there was considerable polymorphonuclear infiltration, and numerous areas of hepatic necrosis were present. The pancreas and the adrenal glands showed no changes. The spleen disclosed complete loss of normal-appearing reticular tissue in the pulp. No unaltered lymphoid tissue was seen, and the structure was obliterated by the tumor, with areas of hemorrhage, infarction and ex-

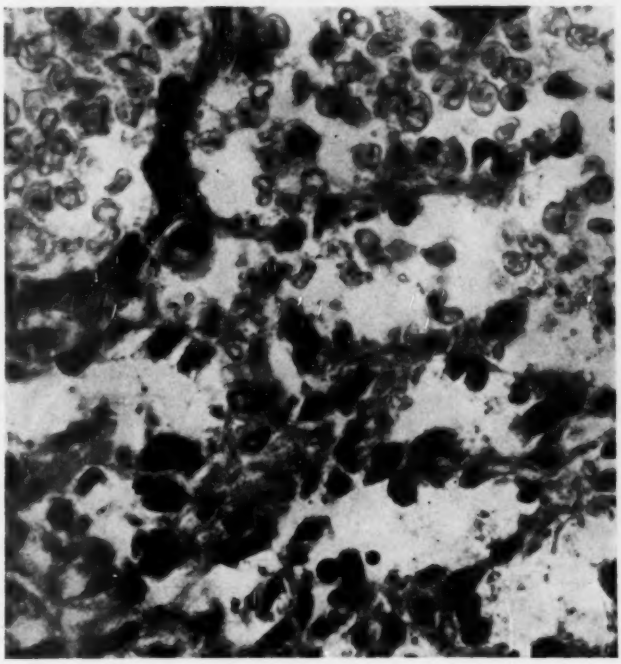

Fig. 2.—Higher magnification of the neoplasm showing atypical endothelial cells with hyperchromatic nuclei lining vascular channels that contain large numbers of red cells; × 875.

tensive necrosis. In places the tumor was extensively infiltrated by polymorphonuclear leukocytes. Wherever found the neoplasm appeared to have as its dominant characteristic the tendency to form slender elliptic channels and somewhat irregular round cavities, which contained blood cells (fig. 1). These channels were lined by plump atypical endothelial cells with hyperchromatic nuclei and rare mitotic figures. (fig. 2). These cells were in a fine fibrous stroma, which showed considerable variation in different areas. In some portions there appeared little tendency for the vascular elements to become differentiated. Here the cells resembled fibroblasts, and poorly defined vascular channels were observed. Rarely,

projecting papillary processes were found. Sections from parietal and visceral peritoneum showed tumors which were similar in all respects to those seen in the liver and the spleen.

Both ribs and vertebral bodies showed angioma with large areas of transition leading to the area of cells which could not be distinguished from fibrosarcoma (fig. 3). Mitoses were infrequent. The marrow was hyperplastic in portions not invaded by tumor.

Postmortem Diagnosis.—Hemangiosarcoma of the spleen with metastases in the liver retroperitoneal lymph nodes, the parietal and visceral pleura, ribs and verte-

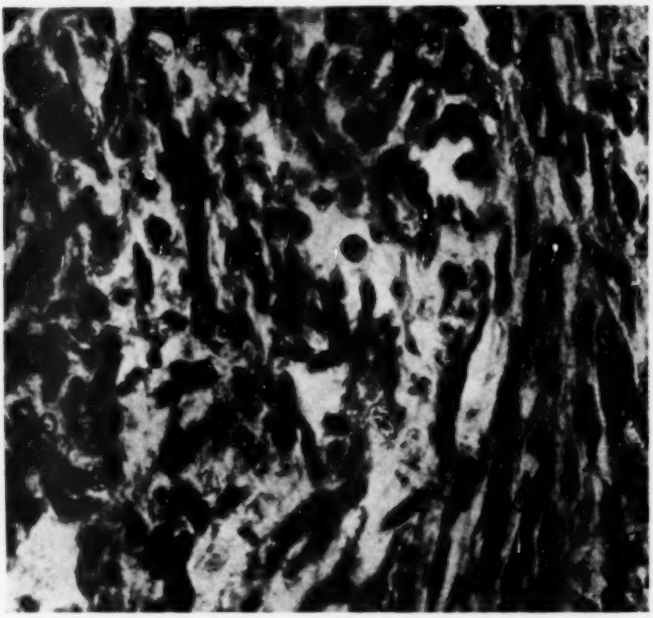

Fig. 3.—A tumor area resembling fibrosarcoma; × 875.

bral bodies; prostatic hyperplasia; stenosis of the right ureter and hydronephrosis; arteriosclerosis; diverticula of the jejunum; Meckel's diverticulum; bilateral pleural effusions.

PREVIOUSLY REPORTED CASES

Case 1 (Langhans[3]).—A 30 year old man with a history of trauma three months before death presented a pulsating, rapidly growing tumor in the left upper quadrant of the abdomen, with dyspnea and abdominal pain. The spleen measured 23 by 15 by 10 cm. at autopsy. Its surface was nodular, and vessels were observed forming cavernous spaces. Microscopically, hyperplastic endothelium

3. Langhans, T.: Virchows Arch. f. path. Anat. **75**:273, 1879.

which was continuous with the sinusoidal endothelium was observed. The cells were described as polyhedral in form. Fibrous and lymphoid tissues did not appear proliferated. A similar growth was found in the liver.

Langhans suggested the possibility of a primary tumor of both liver and spleen but reported it as a primary angiosarcoma of the spleen.

The possibility of a multicentric growth cannot be ruled out. Grossly and histologically it resembled the tumor reported here. Theile[4] and Wright[2] regarded it as similar in all respects to the tumors described by them.

CASE 2. (Theile[4]).—Four angiomatous tumors were reported. One was a cancer occurring in a 56 year old man who died of hemorrhage following splenectomy. The spleen weighed 2,500 Gm. and measured 25 by 16 by 8 cm. There were adhesions and hemorrhagic areas where angiomatous portions extended to the surface. The splenic tissue was replaced by white areas in the pulp, vascular spaces and numerous blood clots. It was found to consist of a network of cavities containing blood and masses of endothelial cells that appeared like "vessel sprouts." Angiomatous areas showed transition to sarcomatous portions with spindle-shaped or round cells and numerous mitotic figures. Metastases were found in the lung, the stomach and the liver.

Theile's case resembled Wright's and mine in that the tumor formed vessel sprouts and showed areas of rapid growth, anaplasia and numerous mitotic figures. Theile assumed an anaplastic proliferation of angioblasts and was justified in calling this tumor sarcomatous angioma. He believed that it was not hemangiosarcoma, since the sheets of spindle cells contained no red blood cells.

CASE 3 (Jores[5]).—A 45 year old woman died of cardiac failure. Hepatomegaly and splenomegaly had been noted eleven months prior to death. A hemogram showed leukopenia. The spleen weighed 3.6 Kg. and measured 31 by 15 by 16 cm. The surface was scarred, and there was obliteration of the splenic structure by a soft, structureless reddish gray mass. A hemorrhagic area was found at the upper pole. Radiation necrosis was prominent. Examination revealed a meshlike tissue with small connective tissue bridges lined with endothelial cells and containing red blood corpuscles. Jores felt that a transition zone was demonstrable from angiomatous to sarcomatous areas. The liver weighed 8.6 Kg. and contained numerous dark periportal metastatic nodules resembling the splenic tumor.

Jores was convinced that the splenic tumor was primary. He pointed out that it was the the first to appear clinically, and that the lesion of the liver was unlike primary angioma of that organ. Furthermore, it showed a distinct periportal distribution which suggested hematogenous spread from the spleen. Jores expressed the belief that this tumor was similar to those of Langhans and Theile. According to Wright, it was similar to the one he reported. This tumor showed a greater tendency toward angiomatous growth than that of Theile and more distinct metastases than that of Langhans. Dispersed red blood cells and a supporting connective tissue wall for endothelial cells with varying grades of differentiation makes this case resemble the case herein reported.

CASE 4 (Wright[2]).—A 25 year old man complained of pain and abdominal swelling of four weeks' duration. There were abdominal distention and epigastric tenderness. There was a leukocyte count of 14,000 with a normal differential count. There was no other alteration from normal in the hemogram. Bile was present in the urine, and clinical jaundice was noted three days after admission.

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4. Theile, F. S.: Virchows Arch. f. path. Anat. **178**:296, 1904.
   Jores, L.: Zentralbl. f. allg. Path. u. path. Anat. **19**:662, 1908.

At laparotomy the spleen and the liver were seen to be greatly enlarged. The patient died postoperatively. The spleen weighed 520 Gm. and measured 15 by 5 by 8 cm. Clotted blood and dark reddish nodules were noted on the surface. A pedunculated spherical tumor was found fixed to the spleen. This mass was composed of large cavities filled with dark bloody material. Sections revealed many small nodular hemorrhagic tumors. The liver weighed 4,250 Gm. and the capsular surface was studded with raised nodular masses which resembled the splenic tumors. The parenchyma was infiltrated with similar masses. Examination of the splenic tumors revealed a neoplasm characterized by the formation of narrow channels filled with blood. The endothelial lining of these channels was atypical and undifferentiated. Projecting papillary processes were observed in the vascular spaces, and from these typical tumor cells were seen to grow. Numbers of red blood cells were seen within the vascular spaces. There was variation in the degree of anaplasia; mitoses were numerous, and areas of rapid growth were described. The lesions of the liver resembled those of the spleen, and their distribution was periportal.

This tumor resembled the one reported here in its tendency to form vascular spaces. It was, however, a more rapidly growing neoplasm. As pointed out by Wright, it is like the tumor reported by Theile.

Case 5 (Paine[6]).—The patient was a 64 year old white man with a sixteen weeks' complaint of dyspnea, lassitude and left-sided abdominal pain. A hemogram suggestive of pernicious anemia was reported. The patient died in cardiac failure. At autopsy the liver (2,400 Gm.) was congested and was studded with small dark nodules. The spleen weighed 1,380 Gm. The surface was thickened by perisplenitis and adhesions. The surface was dull yellowish red, and areas of hemorrhage were observed. A metastatic lesion was found in the left femur. Microscopic examination showed a splenic tumor composed of cells varying from long spindle-shaped cells with deeply hematophilic nuclei to nearly circular cells with vesicular nuclei, some of which bore close resemblance to endothelial cells. Mitoses were rare. Red blood cells were observed throughout the tumor. Lesions in the liver and the marrow were similar.

Paine felt that the tumor was of endothelial origin because there was evidence of phagocytosis in the tumor cells, because there was a tendency to form tubules and because fibrils were seen developing from the cells.

Case 6 (Böckelmann[7]).—A 1½ year old child who had nine hemangiomas removed from the skin and the mucous membranes had the spleen removed for tumorous swelling. A large reddish yellow angiomatous tumor was found. Microscopic examination revealed large endothelial cells lining vascular spaces, and some definitely sarcomatous and angiomatous areas. No metastases were found.

In view of the early age of the patient and the possibility that young proliferating mesenchymal tissue may be confused with sarcoma, it is questionable that this case should be included, although it possesses many characteristics exhibited by the present tumor.

The descriptions provided by Albrecht[8] and Führer[9] are incomplete and are not included. The case reported by Snodgrass,[10] which appears to have been

6. Paine, C. G.: J. Path. & Bact. **34**:139, 1931.
7. Böckelmann, T. W. A.: Ueber ein Angiom der Milz, Dissert., Griefswald, H. Adler, 1906.
8. Albrecht: Ztschr. f. Heilk. **15**:81, 1856.
9. Führer: Arch. f. phys. Heilk. **15**:81, 1856.
10. Snodgrass, T. J.: Surgery **15**:988, 1944.

one of periendothelioma, and that of Ernst,[11] which was an instance of an eruptive angiomatous tumor, are not included because they resemble only remotely the tumors included in this report.

COMMENT

The question of metastases derived from angioma has received considerable attention in the literature (Willis[12] and Pines and Rabinovitch[13]). Ewing[14] established that in some instances angioma may be multicentric, without metastases entering the picture. This is demonstrated in reports of multiple tumors in young persons in whom angioma showed a systemic distribution (Schmitt,[15] Jaffé[16] and Wollstein[17]) or in whom the possibility of a diffuse disease of the reticuloendothelial system was considered (Schlopsnies[18] and Eigler[19]).

In the cases collected here the exclusion of tumors of multicentric origin was not difficult. A primary splenic tumor was observed in cases 1, 2, 3, 4 and 5 and in the case reported. The dissemination of the tumor to include more than one systemic form of distribution is suggested by the location of the metastases in cases 2, 4, 5, 6 and the case which I have reported. The weight of the evidence leads one to presume that in each of these cases the metastases were blood borne from a primary splenic neoplasm.

The latitude of terminology in classifications of primary splenic cancers shows that these tumors present particular difficulties from the morphologic point of view and that they do not fit into the pattern of nomenclature accepted for tumors of other organs. This may be due to the cytologic variations observed in endothelial cells when these occur in tumors of lymphoid organs (Ewing[14]). It may also be attributed to the infrequency with which these tumors are seen by any one observer.

Weichselbaum[20] considered splenic sarcoma as of three types: spindle cell sarcoma, endothelial sarcoma and lymphosarcoma. In 1923 Smith and Rush[21] broadened this initial classification by dividing all splenic

11. Ernst, P.: Verhandl. d. deutsch. path. Gesellsch. **15**:232, 1912.

12. Willis, R. A.: Spread of Tumors in the Human Body, London, J. & A. Churchill, 1934, pp. 148-151.

13. Pines, B., and Rabinovitch, F.: Arch. Path. **33**:487, 1942.

14. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders, Company, 1940.

15. Schmitt, L.: Centralbl. f. allg. Path. **145**:27, 1916.

16. Jaffé, R. H.: Arch. Path. **7**:44, 1929.

17. Wollstein, M.: Arch. Path. **12**:562, 1931.

18. Schlopsnies, W.: Virchows Arch. f. path. Anat. **85**:274, 1881.

19. Eigler, G.: Ztschr. f. Kreislaufforsch. **22**:249, 1900.

20. Weichselbaum, A.: Virchows Arch. f. path. Anat. **85**:554, 1881.

21. Smith, C. E., and Rush, G. Y.: Arch. Surg. **7**:371, 1923.

neoplasms into derivatives of capsule or trabeculae, derivatives of lymphoid elements and derivatives of vascular constituents. As Bostick[1] pointed out, splenic tumors probably should be classified in accordance with the different cell types found in that organ. He listed seven basic neoplastic types.

From Bostick's[1] discussion, however, it is not clear whether he meant to classify all cancerous vascular tumors under the loose heading of angioendothelioma, or whether he would admit cancers in which the unit appeared to be the vessel rather than the cell, angiosarcoma in fact, as a subdivision of this first group. One might question whether such a distinction can or should be made in a group of tumors in which the classic characteristics are ill defined. It is important, as Wright[2] pointed out, to recognize the fact that there exists a group of primary splenic neoplasms which show clearcut differentiation toward well defined vessel formation, i.e., tumors in which the vessels appear to constitute the units of the tumor, and in which many characteristics of sarcomatous tissue are manifested in the morphologic aspects of the tumor cells. This type might well be regarded as separate and distinct from the more commonly described hemangioendothelioma in which the unit appears to be the endothelial cell, vascular spaces are poorly defined, and the tumor cells tend to appear in sheets and masses, characteristic of endothelioma.

In attempting to analyze these tumors from the point of view of their origin and composition, one is confronted with the resemblance they bear both to endothelioma and to sarcoma. Their hemangiomatous properties are evident on gross examination. Microscopically, the tumor is composed of newly formed vessels which encompass fairly large numbers of red blood cells.

Their resemblance to tumors of endothelial origin is manifested by their similarity to Connor's[22] first type of endothelioma in which there is a differentiation toward vascular tissue with sinus formation and the production of vascular spaces. The small cuboidal cells which line the vascular spaces are endothelial cells and the intimate relationship noted between these cells and the supporting stroma is suggestive of the endothelial origin of these tumors.

These tumors cannot be separated from sarcoma in that they reproduce functioning blood vessels. Spindle cells have been described as numerous, as well as many cells which might be considered angioblastic. The nuclei of the sarcoma-like portions of the tumor exhibit in size and form the wide variations characteristic of sarcoma. One might regard this property as consistent with the dual tendency of endothelial cells in lymphoid structures to show epithelial qualities and the potentialities

22. Connor, C. L.: Arch. Surg. **12**:789 (April) 1926.

of connective tissue with reticulum formation penetrating the cell masses and outlining the vessel walls. On the other hand, the connective tissue framework of the developing blood vessels and the absence of sheets of endothelial cells incline to the diagnosis of sarcoma.

The term "angiosarcoma" has been used to designate all tumors originating from blood and lymph vessels and might be applied to these tumors. The subdividing of angiosarcoma into perithelioma (Hildebrandt[24]) and periendothelioma (Borrmann[25]) does not apply to this tumor, since the tumor cells neither encircle nor radiate from the developing vessels.

In my opinion they should be regarded as capillary hemangiosarcoma until such time as the differentiation between endothelioma and sarcoma can be made with certainty and the exact histogenesis of the constituent cells be established.

An incidental finding of importance, and one which has received little attention in the literature, is that alterations occur in the hemograms of patients with splenic cancer. (Cases of Howard,[26] Frank,[27] McNee,[28] Shennan[29] and Paine[6] and my own case.) In the reported hemograms one notes morphologic changes in the red blood cells which are suggestive of pernicious anemia, i.e., anisocytosis, poikilocytosis, polychromasia and occasional nucleated red blood cells with leukopenia and thrombopenia. This resemblance occurring without the neurologic changes or the glossitis of pernicious anemia and with or without achlorhydria was pointed out by Howard in reporting his case of splenic lymphosarcoma. These changes have been reported frequently enough in cases of primary splenic cancer to be of diagnostic aid in cases of suspected splenic tumor. Both Matas[30] and Grove[31] implied that hemangiomatous growths increase splenic function; whereas, in a case such as mine it would appear that a tumor replacing, invading and compressing normal splenic pulp would tend to decrease rather than to increase splenic function. The exact relationship of the changes of the hemogram

23. Footnote deleted by the author.

24. Hildebrandt, C. L.: Deutsche Ztschr. f. Chir. **31**:263, 1891.

25. Borrmann, C. L.: Virchows Arch. f. path. Anat. **157**:297, 1899.

26. Howard, T.: J. Lab. & Clin. Med. **14**:1157, 1929.

27. Frank, L.: Am. J. M. Sc. **183**:77, 1932.

28. McNee, J. W.: J. Path. & Bact. **39**:83, 1934.

29. Shennan: J. Path. & Bact. **15**:139, 1914.

30. Matas, R., in Piersol, G. M., and Bortz, E. L.: Encyclopedia of Medicine, Philadelphia, F. A. Davis Company, 1934, vol. 12, p. 843.

31. Groves, E. W. H., cited by Campbell, W. C., in Piersol, G. M., and Bortz, E. L.: Encyclopedia of Medicine, Philadelphia, F. A. Davis Company, 1934, vol. 7, p. 640.

and splenic cancer cannot be explained in the present knowledge of splenic function. Furthermore, the changes do not seem to be related to the cell type of the invading tumor, since the reported cases are of several histologic varieties.

SUMMARY

The capillary hemangiosarcoma of the spleen presented here is one of a small group of rare primary splenic neoplasms which are characterized by formation of vascular channels, by areas of sarcomatous aspect morphologically and by metastases. It was associated with changes in the hemogram suggestive of pernicious anemia, and terminated the life of the patient within four years after the onset of symptoms which, in retrospect, were suggestive of splenic involvement.

CONGENITAL RHABDOMYOMATOSIS OF THE HEART

Report of a Case with Autopsy

AMBROSE J. HERTZOG, M.D.
MINNEAPOLIS

CASES of the clinical syndrome of congenital idiopathic cardiac hypertrophy can be separated as a rule into four main groups. The first and most common are those in which the hypertrophy is associated with endocardial sclerosis and myocardial fibrosis. A second group includes those rare cases in which the enlargement is due to true hypertrophy of the muscle fibers. Glycogen storage disease of von Gierke accounts for the third group. A remarkable feature is the unusual stability of the glycogen stored in the liver and other tissues, regardless of fixation and time after death. The fourth rarest cause of this syndrome is found in those cases in which the enlargement of the heart is due to localized tumors or a diffuse tumor of the heart composed of pale-staining, vacuolated cells resembling embryonic muscle fibers. A variety of names have been used in recent years to describe these tumors. The earlier authors described them as rhabdomyoma. Von Recklinghausen[1] is generally credited with reporting the first case, in 1862. Some of the early cases were reported by Knox and Schorer[2] in 1906 and Wolbach[3] in 1907. In more recent years cases have been reported by Farber,[4] Yater,[5] Ill and Gray,[6] Wegman and Egbert,[7] Olsen and Cooper,[8] Hillman,[9] Batchelor and Maun[10] and Haymond and Giordano.[11] Batchelor and Maun[10]

From the University of Minnesota Teaching Service of Northwestern Hospital and the Department of Pathology of the University of Minnesota Medical School, Minneapolis.

1. von Recklinghausen: Monatschr. f. Geburtsch. **20**:1, 1862.
2. Knox, J. H. M., and Schorer, E. H.: Arch. Pediat. **23**:561, 1906.
3. Wolbach, S. B.: J. M. Research **16**:495, 1907.
4. Farber, S.: Am. J. Path. **7**:105, 1931.
5. Yater, W. M.: Arch. Int. Med. **48**:627, 1931.
6. Ill, C. H., and Gray, F. W.: Am. J. Obst. & Gynec. **28**:264, 1934.
7. Wegman, M. E., and Egbert, D. S.: J. Pediat. **6**:818, 1935.
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made a thorough review of the literature in 1946 and collected 63 cases. They preferred the term "glycogenic tumors of the heart." Most of the recent articles have all stressed the glycogen content of the tumors. However, in the greater majority of the reported cases attempts to demonstrate glycogen in the hearts were unsuccessful. This failure has been attributed largely to lack of alcoholic fixation of the tissues. In 35 or 65 cases collected from the general literature, including our own, the patients were children under 1 year of age. Only in 7 cases were the patients children over 15 years of age.

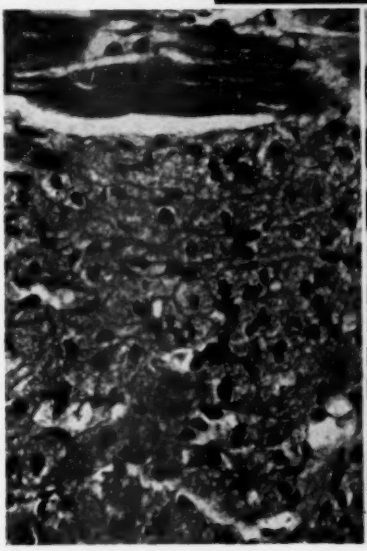
The case now reported is to my knowledge the first encountered in the department of pathology of the University of Minnesota in over 52,000 autopsies. It is of interest because of the failure to demonstrate glycogen in the tumor cells after alcoholic fixation.

REPORT OF CASE

A girl was born at Northwestern Hospital on Sept. 10, 1947. She weighed 5 pounds and 10 ounces (2,551.5 Gm.) at birth. The delivery was normal. It was the mother's second pregnancy. The first child was living and well. The newborn baby appeared normal during the first few days of life. There was no cyanosis. She failed to gain weight and ate poorly. Her weight on leaving the hospital on the sixth postnatal day was 5 pounds and 10 ounces. She was readmitted October 24, at the age of 6½ weeks, to the service of Drs. Erling Platou and Richard Tudor, because of an infection of the upper respiratory tract. She was poorly nourished. The examination otherwise disclosed no abnormality. She showed some gain in weight and was discharged as improved on October 28. Her appetite remained poor. On November 11 the mother noted that the baby's breathing was irregular and that she could not be aroused. She was brought again to the hospital on November 11 at 4:30 a.m., cyanotic and comatose. Her temperature was 97.8 F. rectally; the pulse rate was 140 per minute, and the respirations were irregular and shallow at 15 to 20 per minute. There was intrasternal and suprasternal retraction of the chest. The breath sounds were faint. The resonance of the chest was increased. The cardiac rate and rhythm were regular. Murmurs were not heard. The heart appeared enlarged. The edge of the liver was down 5 cm. below the right costal margin. The baby was limp, with poor muscular tone. A roentgenogram of the chest showed diffuse cardiac enlargement, the heart occupying most of the thoracic cavity. The baby was given oxygen, caffeine, sodium benzoate, epinephrine hydrochloride and adrenal cortex extract. Laryngoscopic examination showed the upper respiratory passages to be clear. The baby was given 1 cat unit of lanatosid C (cedilanid^{R}) and treated with a penicillin spray, but she remained comatose and died November 11, at the age of 2 months.

Autopsy (four and a half hours after death).—The body was that of a poorly nourished, well developed white girl, measuring 53 cm. in length and weighing 2,880 Gm. Edema, jaundice and rigor were absent. There was cyanosis of the lips and the finger tips.

The peritoneal cavity was empty, and the surfaces were smooth. The edge of the liver was down 3 cm. below the right costal edge and 4.5 cm. below the xiphoid process. Each pleural cavity contained about 7 cc. of clear straw-colored fluid. The pericardial sac appeared normal. The transverse cardiac diameter was 8 cm.,



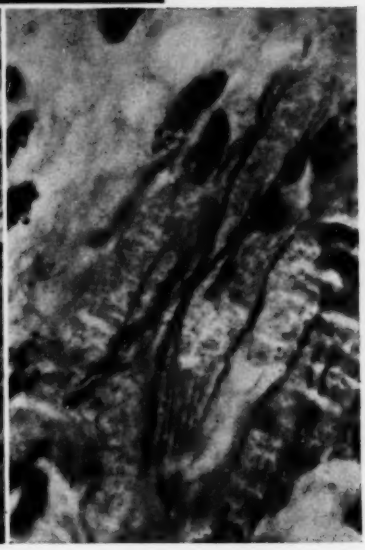


Fig. 1.—Heart with nodular elevations on the anterior surface of the left ventricle.

Fig. 2.—Photomicrograph in which the pale, granular cells of the tumor may be compared with normal cardiac muscle.

Fig. 3.—Photomicrograph showing cross striations in tumor cells; iron-hematoxylin stain.

compared with a transverse thoracic diameter of 9.3 cm. The heart filled the greater part of the thoracic cavity.

The heart weighed 66 Gm. The anterior surface of the left ventricle was covered by several flat, ill defined yellowish brown tumors measuring up to 1.5 cm. in size (fig. 1). Both ventricles were involved by yellowish brown, irregular tumors, but most of the tumors were confined to the left ventricle. The auricles appeared normal. The appendages and the values appeared normal. The endocardium, particularly that of the left ventricle, was pale. The arch of the aorta appeared normal. The ductus arteriosus was closed. The coronary orifices and vessels appeared normal.

The lungs were dark red, and crepitation was slightly decreased. The right lung weighed 27 Gm. and the left 20 Gm. The spleen weighed 9 Gm. and appeared normal. The liver weighed 115 Gm. Its color was a homogeneous yellowish brown. The gallbladder, the gastrointestinal tract, the pancreas and the adrenal glands appeared normal. The kidneys weighed 15 Gm. each and showed nothing of note. The bladder and the genital organs presented no anomalies. The thymus weighed 21 Gm. The aorta was smooth. Permission to examine the brain was not obtained.

Microscopic Examination.—Sections of the heart stained with hematoxylin and eosin showed the myocardium of the septum and of both ventricles to contain circumscribed tumors composed of large, pale, granular cells. The cells were much larger than the normal muscle fibers (fig. 2). The cell boundaries were indistinct. The tumor cells contracted strikingly with the normal muscle. Iron-hematoxylin stains showed some of the tumor cells to contain distinct cross striations (fig. 3). Best's carmine stain on heart muscle fixed in absolute alcohol at the time of autopsy showed absence of glycogen in the tumor cells. Frozen sections of the heart muscle stained with sudan III showed that the tumor cells contained fat droplets within their cytoplasm.

Best's carmine stain on sections of the liver fixed in absolute alcohol at the time of autopsy showed a normal amount of glycogen in the liver cord cells in the centers of the lobules. The liver appeared normal in sections stained with hematoxylin and eosin. Section of the remaining organs revealed nothing of note.

COMMENT

Whether these tumors represent true neoplasms, developmental anomalies, metabolic disturbances or degenerative phenomena has been a point of discussion since the first report of a case was published. Wolbach[3] considered the tumor a true neoplasm as a result of the demonstration of early intracellular fibril formation. Many have stressed the fact that the large, pale, vacuolated cells resemble embryonic muscle. The separation between the various types of hyperplasia and neoplasm is so indefinite that one cannot say whether these tumors should be described as neoplasms. The term "glycogenic tumors of the heart" appears to be a poor one. Batchelor and Maun[10] stated that glycogen was demonstrated in only 8 of the 63 cases collected from the literature. The fact that rhabdomyoma of the heart is associated, in approximately 50 per cent of cases, with tuberous sclerosis seems particularly significant. In many of the other cases, cysts and renal tumors, tumors of sebaceous glands, harelip, cleft

palate, multiple glioma of the brain and other malformations were recorded. Hueper[12] considered this an evidence that congenital rhabdomyoma of the heart represents a partial manifestation of a generalized developmental disturbance affecting various organs of the body. The tumors of the heart could be best described as a developmental disturbance, or hamartoma. In none of the reported cases was evidence of continuous neoplastic growth or ability to metastasize ever shown. There seems to be little to support the hypothesis that the tumors represent degenerative phenomena of the heart muscle. The term "rhabdomyoma" or "rhabdomyomatosis" is still to be preferred to those linking the tumors to disturbances of glycogen metabolism. The tumors bear considerable histologic similarity to myoblastoma occurring in other portions of the body.

SUMMARY

In a 2 month old infant dying of heart failure, autopsy showed diffuse rhabdomyomatosis of the heart. The tumors are interpreted as hamartoma.

Touro Infirmary.

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BASAL CELL CARCINOMA WITH METASTASES

A Report of Two Cases

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BASAL cell carcinoma, though frequent, rarely metastasizes, even to nearby lymph nodes. Cases in which metastases are found in distant organs are therefore worthy of note.

Nine such cases have been reported: Körbl[1] reported a case in which massive invasion of cervical lymph nodes occurred seventeen months after treatment of a primary basal cell carcinoma of the right temple. Beadles[2] observed, in a 46 year old man, a primary tumor of the face with metastases in submaxillary lymph nodes. Finnerud[3] presented 2 cases. In one a 39 year old man had a tumor of thirteen years' duration on the face, which repeatedly metastasized to cervical lymph nodes; in the other a 56 year old man with primary basal cell carcinoma of the cheek of thirty years' duration showed spreading of the tumor, at that late date, to submaxillary nodes. Eller[4] presented before the Manhattan Dermatological Society a 37 year old man who three years previously had a sore on one finger of the right hand, which was diagnosed as "epithelioma from inner sheath of hair shaft." The finger was amputated, and the histologic diagnosis was "basal cell carcinoma." Twenty-five months later a lump was removed from the right axilla, which the pathologist called "advanced squamous cell carcinoma." A year later, three lesions appeared on the forearm, two of which yielded biopsy specimens which were diagnosed as "typical basal cell carcinoma." On reviewing the axillary lymph node, the pathologist called it "basal cell carcinoma." Forty-five months after the first biopsy,[5] the man was presented again to the aforenamed society, still alive but with a badly swollen arm. Niles[6] reported the death of this patient after amputation of the arm and recurrence of the tumor in the supraclavicular lymph nodes. No autopsy was done.

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De Navasquez[7] recorded the case of a 44 year old white woman with a "pea-sized" nodule in the skin of the midforehead. On three occasions it was incised, and only after five and one-half years was it removed, having attained a size of 2.5 by 2 cm. At that time it was fixed to both skin and bone. The histologic diagnosis was "basal cell carcinoma." A course of roentgen therapy was given. Eight months later the patient complained of bone pain and was very anemic. She died six years and four months

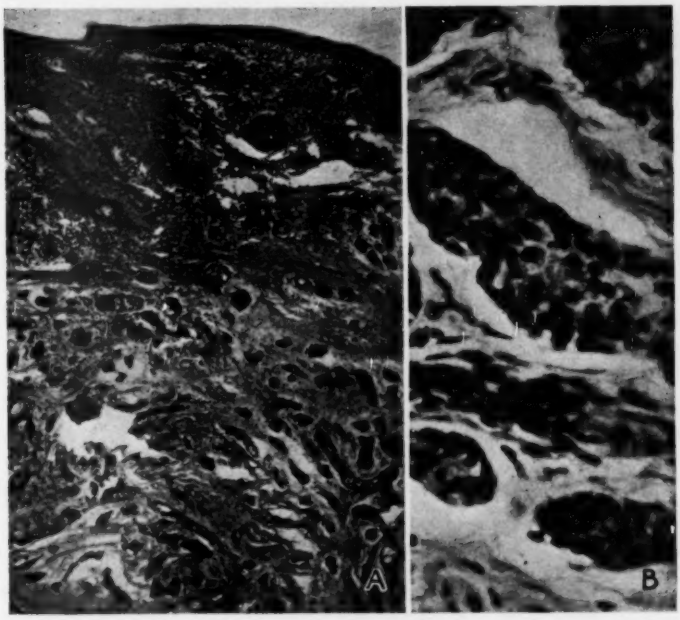

Fig. 1 (case 1).—*A*, biopsy specimen of ear showing invading basal cell masses beneath intact epidermis; × 40. *B*, higher magnification of an area of *A* showing uniform hyperchromatic basal cells; × 300.

after the tumor first appeared in the skin of the forehead. Autopsy showed a neoplasm typical of basal cell carcinoma, metastatic to bones and lungs.

Two instances were reported by Spies.[8] One patient was a 58 year old white man, who had a 2 mm. tumor in the right nasolabial fold, twice treated with radium, but recurrent again. On surgical removal it proved to be basal cell carcinoma. It recurred three months later, and at autopsy metastases were found in ribs, spleen and liver. The second patient, a 50 year old white woman, had a tumor of the nasal mucosa, which gave rise to an epigastric metastasis diagnosed as basal cell carcinoma. At autopsy

7. De Navasquez, S.: J. Path. & Bact. **53**:437, 1941.

8. Spies, J. W.: Arch. Surg. **21**:365 (Sept.) 1930.

basal cell growths were found in spleen, peritoneum, lungs, ribs and femur.

REPORT OF CASES

CASE 1.—J. I., a white man 37 years old, was admitted to a hospital, March 4, 1938, with "malignancy of the left ear." In 1931 a "cystlike tumor" of the left

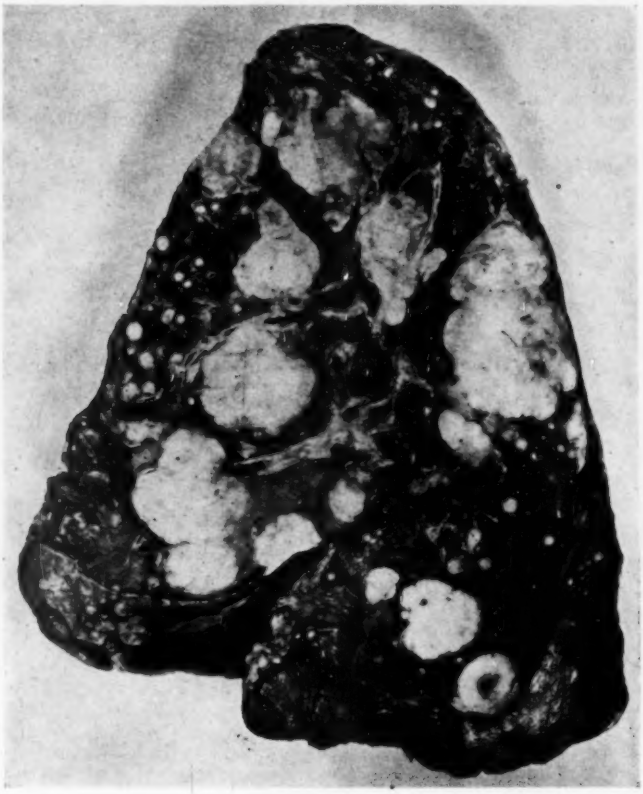

Fig. 2 (case 1).—Lung showing tumor metastases.

external auditory meatus was removed. It was not examined microscopically. In 1933 a spreading ulcer developed in the wound. After removal of the ulcerous area, the skin was smooth for eighteen months, but in 1935 a new ulcer appeared. Two more recurrences followed inadequate removals.

In November 1937 he noted numbness and weakness of the left side of the face, with "fulness" in the ear. The next month he had vertigo, nausea and

vomiting. There was severe pain in the left ear and in the chest. He had lost 40 pounds (18.2 Kg.) in six months and was weak.

Examination revealed a weakened, ill nourished white man. The left tragus was missing, the concha and the lobe of the ear were distorted and partly missing, and the auditory meatus was obliterated. The tissue near the meatus was indurated and ulcerated. Two firm nodes were left 2 cm. below the tip of the mastoid process. There was complete paralysis of the left side of the face. Some generalized abdominal tenderness was present.

Biopsy of the ulcerated area showed basal cell carcinoma (fig. 1*A* and *B*.)

Roentgenograms revealed marked increase of density in the left mastoid process, any many rounded densities 1 to 4 cm. in diameter in both lung fields.

Since basal cell carcinoma is but rarely responsible for visceral metastases, another primary source was sought. Physical examination of nasopharynx, mouth, larynx, neck, rectum (by sigmoidoscope) and bladder (by cystoscope) disclosed no tumor. Roentgenologic study of the whole digestive system revealed no evidence of involvement. Pyelograms showed no renal lesion.

Roentgen therapy to the region of the left ear and to the chest produced remission of the nausea and vomiting and the pain, and the patient left the hospital May 11, 1938.

Oct. 14, 1938 he felt well; he had regained his previous weight, and the ulceration of the external ear was healed, though four fifths of the ear was missing. Roentgenograms showed 50 per cent shrinkage of the pulmonary growths.

March 10, 1939 he noted pain in the upper abdominal region and a loss of 4 pounds (1.8 Kg.) in weight. A repetition of the search for a second primary tumor was fruitless.

He died Aug. 1, 1939, eight years after the first tumor appeared on his left ear.

Autopsy.—The body was somewhat emaciated. The left external ear was represented by only a few stubs covered with smooth skin.

The pericardium presented on its outer surface a few 1 cm. firm white plaques of tumor. The heart showed no lesion.

The lungs adhered by a few weak bands to the parietal pleura which, on both lateral and diaphragmatic surfaces, was studded with great numbers of 2 mm. to 2 cm. white tumor nodules. The lungs weighed 1,000 Gm. each and displayed numerous masses of pale yellow, slightly spongy tumor, the largest 5 cm. in diameter (fig. 2). The main bronchus of the upper lobe of the right lung was occluded in its upper part by a granular pale gray mass which was thought at first to be a primary bronchial carcinoma. A 1 cm. abscess lay just distal to this occlusion. The bronchi presented no other lesions. The lung tissue proper was edematous.

The peritoneum and the mesentery, like the pleura, were the site of tumor nodules up to 2 cm. across. The intestines appeared unchanged throughout.

The liver weighed 3,000 Gm., and on its surface were several tumors, the largest 8 by 6 cm. The hepatic tissue was passively congested.

In the spleen, which was congested and which weighed 350 Gm., was found a single 1 cm. white round tumor. The tracheobronchial and periaortic lymph nodes and one small node near the tail of the pancreas were enlarged by firm tumor. There were two small accessory spleens.

Each kidney contained several tumor nodules, averaging 1 cm in diameter.

Since a tumor had been found in one bronchus, it was suspected that all the tumors elsewhere were metastases of that one. Microscopic study, however, led to a different conclusion.

Histologic Examination.—The tumor in all its locations (fig. 3*A* and *B*), including the affected bronchus, was composed of invasive groups of small oval cells with pale cytoplasm and very indistinct cell boundaries and with extremely rare mitotic figures. No resemblance to squamous cells or to glands was seen although rounded spaces filled with mucoid material were common. The tumor resembled basal cell carcinoma with cystic degeneration and was similar to the biopsy specimen of the ear taken seventeen months before death, except that the latter was not cystic. The anatomic diagnosis, therefore, was: basal cell carcinoma

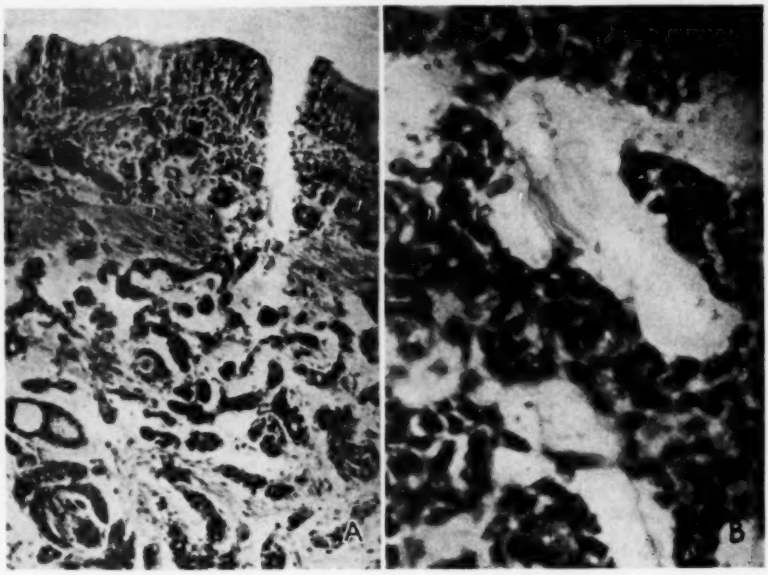

Fig. 3 (case 1).—*A*, bronchus showing tumor in the bronchial wall. Note the cystic formations and the uniformity of the cells; × 60. *B*, higher magnification of an area of *A*; × 300.

of the left ear; pulmonary, hepatic, splenic, renal, pleural, peritoneal, pericardial and lymph node metastases of basal cell carcinoma; pulmonary edema. Permission to open the cranium was not secured.

Case 2.—J. C., a white man aged 62, was seen in the tumor clinic of the San Bernardino County Hospital, Feb. 7, 1938, with a 2 by 3 cm. indurated ulcer of ten years' duration just anterior to and involving the right auditory meatus.

A biopsy specimen of the margin of the ulcer was diagnosed as basal cell carcinoma with some areas suggestive of early squamous cell carcinoma.

March 2 the right ear and the ulcerous area were amputated with the cutting cautery. The specimen showed a 2 by 3 cm. ulcer, microscopic study of which revealed basal cell carcinoma with invasion of the perichondrium of the cartilage of the ear.

Since the amount of skin removed from the face precluded primary closure, the wound was left open, and future skin grafting was contemplated. Roentgen therapy, a total of 6,600 roentgens, was given. Although no tumor recurrence was evident, the wound never healed well. Repeated débridement was done, and finally the temporomandibular joint was removed, but healing was never satisfactory.

Jan. 28, 1939 the patient was seen in the tumor clinic complaining of pain in the chest and the shoulders, and of cough, but no fever. Roentgenograms showed mottling of both lung fields.

Fig. 4 (case 2).—Basal cell carcinoma; × 300.

From this time on he gradually became weak, had persistent pain in the region of the right ear and in the chest, and died Aug. 17, 1939.

Autopsy (forty hours after death).—The body was that of an emaciated elderly white man. The right ear was completely absent, and a 6 cm. ulcer with thick raised margins occupied its site.

The brain weighed 1,400 Gm. and contained no lesion. Beneath the dura of the right middle cranial fossa were a few tiny flakes of firm white tissue.

The lungs showed many 2 to 3 mm. crumbly white tumor nodules, not in proximity to bronchi. There were also two 2 cm. tumor growths near the pleura.

The liver weighed 1,400 Gm. and contained about 50 firm white tumor nodules up to 8 mm. in diameter. The spleen weighed 100 Gm., was firm and

light red, and contained three white tumor nodules about 7 mm. across. The tracheobronchial nodes did not contain grossly obvious tumor, but microscopic sections revealed tumor invasion. No enlargement of the cervical lymph nodes was seen.

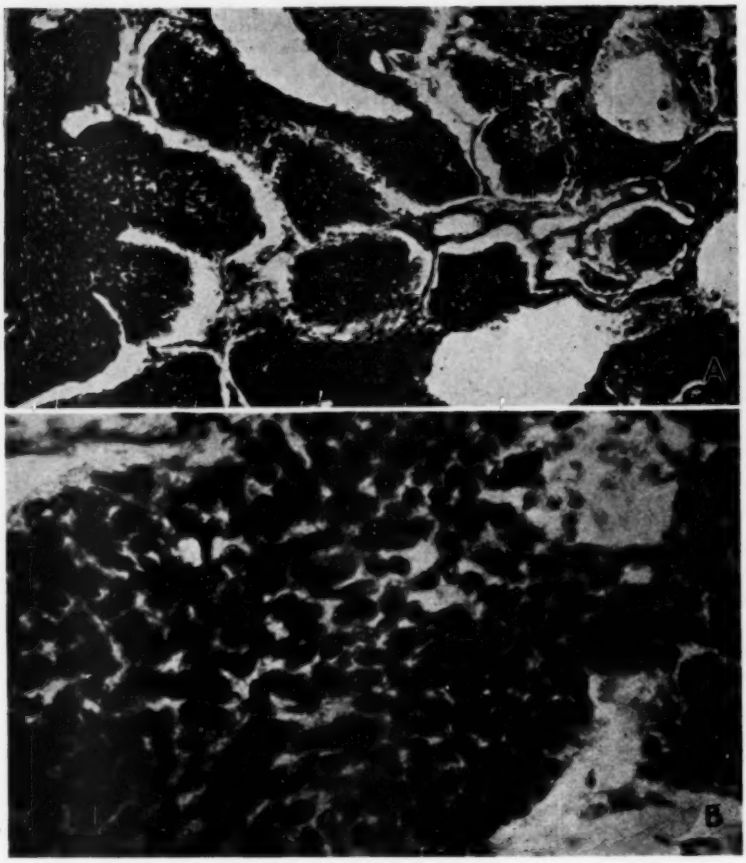

Fig. 5 (case 2).—*A*, lung showing intra-alveolar tumor cell invasion; × 80. *B*, higher magnification of an area of *A*; × 500.

Each kidney weighed 125 Gm., and each contained a few tumor growths up to 1 cm. in diameter. The prostate gland was small and rubbery.

The circulatory and digestive systems revealed no lesion. No tumor or thrombus was seen in the exposed right lateral dural sinus.

Microscopic Observations.—Reexamination of the original biopsy specimen from the ear confirmed the diagnosis of basal cell carcinoma. (fig. 4). The squamous cell elements only remotely suggested neoplasia.

The tumor growths in lungs, liver, spleen, tracheobronchial lymph nodes and kidneys (fig. 5) were composed of sheets and cords of uniform medium-sized oval cells with pale cytoplasm and rather dark nuclei. Mitotic figures were scarce. The cells were similar to those in the antemortem biopsy specimen. The stroma accompanying the tumor was edematous connective tissue. In the tumor areas of the lung the alveoli were not displaced, nor were their walls destroyed; they were simply filled with solid sheets of tumor, suggesting that the tumor had penetrated into a bronchus and spread by extension into the alveoli.

Sections from the thickened margin of the ulcer of the temporal region, however, revealed quite typical squamous cell carcinoma. This recalls the suggestion of squamous cell carcinoma in the original biopsy, though squamous elements were admittedly meager in the biopsy tissue.

The anatomic diagnosis was: basal cell carcinoma of the ear; squamous cell carcinoma of the skin of the region of the ear; metastases of basal cell carcinoma in the lungs, the liver, the spleen and the kidneys.

COMMENT

Case 1.—Since the head was not opened, there may be some suspicion that the primary tumor was an intracranial one. However, metastases of primary intracranial tumors are rare; hence an intracranial tumor would be improbable as the source of the visceral metastases here. Certainly there was some lesion of the vestibular apparatus to account for the vertigo, which was severe enough to prevent the patient's sitting up. What this lesion was we unfortunately do not know. That the tumor of the external ear had either arisen in or extended to the canal of the ear was shown in the scarring and deformity of that region and in the biopsy specimen obtained on the first clinic visit, part of which came from the canal of the ear. The fact that vertigo, nausea and vomiting ceased entirely under roentgen therapy, and that the enlargement of the mastoid region, which regressed under roentgen therapy did not return, and that there were no symptoms of intracranial disease during the last sixteen months of life, is against the idea of the presence of an expanding, or even a persisting, organic lesion of the brain or the internal ear. Indeed, it may be that the vestibular symptoms occurred because of persistent occlusion of the auditory canal. The conclusion, therefore, was that the basal cell carcinoma of the external ear was the primary tumor and gave rise to the other growths.

Case 2.—The diagnosis of the original biopsy specimen as predominately basal cell carcinoma seemed to be beyond dispute. The extent of participation of typical squamous cells in that specimen is quite small.

The principal question to be settled is whether the visceral metastases sprang from a primary tumor arising in a bronchus or from basal cells of the cutaneous tumor. Gross examination did not disclose any primary tumor of a bronchus or of any other site.

The original biopsy specimen and the visceral metastases were distinctly basal cell type. The tumor taken from the temporal region at autopsy was squamous cell type. However, the fact remains that the metastases still resembled the obviously basal cell tumor of the biopsy and bear little similarity to the temporal tissue removed at autopsy. This squamous cell carcinoma must have developed from squamous cell elements in the original tumor.

SUMMARY

Two cases of basal cell carcinoma of the external ear with generalized metastases are presented.

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Litman Oxgall Agar is a new selective medium containing streptomycin for primary isolation of pathogenic fungi. Reference, American Journal of Clinical Pathology, Vol. 18, No. 5, May 1948.

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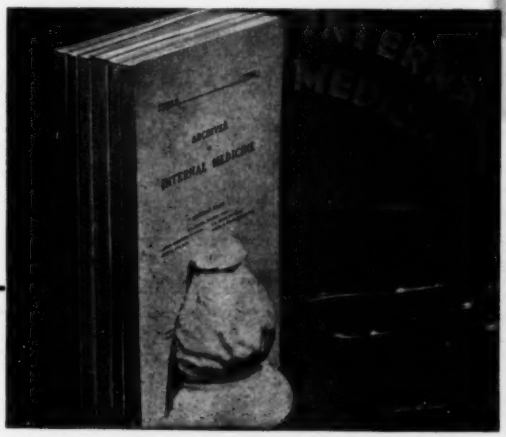

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